EVALUATION OF ANTI-ANAEMIC ACTIVITY OF *Phyllanthus emblica* Linn., ON STREPTOZOTOCIN INDUCED RATS

A dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

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in partial fulfilment of the requirements for the award of the degree of

MASTER OF PHARMACY IN

PHARMACOLOGY

Under the guidance of

Mrs. R. INDUMATHY, M. Pharm.,

Submitted by

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INSTITUTE OF PHARMACOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI-600003

APRIL-2016

CERTIFICATE

This is to certify that this dissertation work entitled **"EVALUATION OF ANTI-ANAEMIC ACTIVITY OF *Phyllanthus emblica* Linn., ON STREPTOZOTOCIN INDUCED RATS"** submitted by **Reg.No. 261426064** in partial fulfilment of the requirements for the award of the degree in **MASTER OF PHARMACY IN PHARMACOLOGY** by the Tamil Nadu Dr.M.G.R. Medical University, Chennai is a bonafide record of the work done by him in the Institute of Pharmacology, Madras Medical College, Chennai during the academic year 2015-2016 under the guidance of **Mrs. R. INDUMATHY, M.Pharm., Asst. Professor**, Institute of Pharmacology, Madras Medical College, Chennai-600003.

Place: Chennai-03

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The Dean,

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Dedicated to my Parents

CONTENTS

LIST OF ABBREVIATIONS

RBC	Red Blood Cell
HB	Haemoglobin
WHO	World Health Organisation
DLHS	District Level Health Service
NFHS	National Family Health Survey
MCV	Mean Cell Volume
MCH	Mean Cell Haemoglobin
IDA	Iron Deficiency Anaemia
CSF	Colony Stimulating Factor
ROS	Reactive Oxygen Species
G6PD	Glucose 6-Phosphate
SOD	Superoxide Dismutase
PRX	Peroxiredoxin
$.O_2$	Superoxide
H_2O_2	Hydrogen Peroxide
.OH	Hydroxyl Radical

XO	Xanthine Oxidase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NOS	Nitric Oxide Synthase
COX	CycloOxyganase
GPx	Glutathione Peroxidise
GSH	Glutathione
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
CRP	C-Reactive Proteins
DLA	Dalton Lymphoma Ascities
LPS	Lipopolysaccharide
HUVEC	Human Umbilical Vein Endothelial Cells
TNF	Tumour Necrosis Factor
TBARs	Thiobarbituric Acid Reactive Species
SA	Sodium Arsenite
MCHC	Mean Cell Haemoglobin Concentration
RDW	Red Cell Distribution Width
HCT	Hematocrit
PCV	Packed Cell Volume
PUFA	Poly Unsaturated Fatty acid

Introduction

1 INTRODUCTION

Anaemia is the blood disorder characterized by reduction in the total number of red blood cells, haemoglobin concentration and packed cell volume[1]. Haemoglobin is a main part of RBC that makes the blood cells red which binds with oxygen. There is an insufficient oxygen supply to the body in case of anaemia. The reduction in Red Blood C ells results in impaired oxygen delivery to tissues, giving rise to physiologic consequences of tissue hypoxia, fatigue, weakness, dizziness, headache, numbness or coldness in hands and feet, low body temperature, pale skin, rapid or irregular heartbeat, shortness of breath, chest pain and irritability[2].

The normal level of haemoglobin is generally different for men and women. For men, anaemia is typically defined as haemoglobin level of less than 13.5g/100ml and in women as haemoglobin of less than 12.0 grams/100ml[3].Anaemia caused by blood loss, decreased red blood cell production, destruction of red blood cell and low haemoglobin level[4].

Statistics

Anaemia is the major health problem and its needs urgent treatment. WHO report stated that, In 1992-the overall prevalence of anaemia is about 42% in developing countries. Among there, 51% are pregnant women and 41% are non-pregnant women. This report stated that half of the women are from the South East Asia are anaemic. In 1993-2015, the overall prevalence is about 25%. It includes 65.5%- preschool children, 48.2%-pregnant women, 45.7%- non pregnant women were estimated to have anaemia. In adulthood female has more prevalence and more severity of anaemia, whereas in children male children had higher prevalence to anaemia than female[5].

In India, anaemia became the major public health challenge that attacks preschool children and women at reproductive age. In 2002-2004-DLHS (District Level Health Service) reported that, 98% of adolescent girls and 96% of pregnant women are suffered from anaemia. In 2005-2006- NFHS (National Family Health Survey) reported that 24% of men are also found to have anaemia due to low level of haemoglobin than normal level. Even though the government carries out several interventions, Anaemia is still remains endemic[5].

1934 George Richards Minot and his assistant, William Parry Murphy: shared the **Nobel Prize** for their discovery about pernicious anaemia could be successfully treated with large quantities of raw liver in patient's diets[6].

WHO definition for anaemia

Anaemia is a condition in which the number of red blood cells or their oxygen carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking and pregnancy status.In its severe form, it is associated with fatigue, weakness, dizziness and drowsiness. Pregnant women and children are particularly vulnerable[7].

CLASSIFICATION

RBC indices are important in the assessment of anaemia.In general, two approaches are used to identify the likely cause of, alterations of the RBC indices and manual examination of a blood smear[2]. The first step in the classification of is to assign the type based on the average size of the red blood cells. If the MCV (Mean Corpuscular Volume) is < 80 fL it is considered microcytic, >100 fL macrocyticand the values between these two are normocytic[8].

Macrocytic anaemia

Abnormal nucleic acid production in the RBC precursor cells such as in B12 and folate defciency result in macrocytosis. When the MCV is greater than 115fL the anaemia is called megaloblastic.

Macrocytosis is a characteristic of juvenile RBC. Thus, often haemolytic anaemias, which are associated with increased production and release of red cells (reticulocytosis) are macrocytic[8].

Microcytic anaemia

This anaemia is most often accompanied by hypochromasia (MCH of less than 27pg).

Normocytic anaemia

This is a rather large and somewhat ill defined group of disorders in which the differential diagnosis can often be narrowed by careful examination of the blood film. Detection of a characteristic morphological abnormality such as tear drop cells, sickle cells, spherocytes [8].

CAUSES OF ANAEMIA

1. Destruction of RBC

It occurs when red blood cells are being destroyed pre maturely. The normal lifespan of RBCs is 120 days; in haemolytic anaemia, it's much shorter and the bone marrow (the soft, spongy tissue inside bones that makes new blood cells) simply cannot keep up with the body's demand for new cells. This can happen for a variety of reasons. Sometimes, infections or certain medications – such as antibiotics or antiseizure medications are also cause anaemia[2].

2. Blood loss

Blood cells naturally die and are reproduced in the body. There are times when the production of the red blood in the body cannot cope with the demand for it. This usually happens during times when there is severe issue of blood like in the case of women having irregular menstruation. Internal bleeding like in the case of people with ulcer also contributes to the abnormal discharge of blood. As a rule, when a red blood cell is destroyed, it should be a replaced. The body is capable of producing red blood cell of its own. Production takes place in the bone marrows. There are times when the bone marrow lacks the ability to produce the right amount of red blood. In this case the red blood cells count decreases, which results in a certain type of anaemia [1].

3. Other causes for anaemia

3.a Iron Deficiency Anaemia (IDA)

As the name implies this type of anaemia is caused by the lack of iron in the body. Iron is used in the production of red blood cells in the body. When a person lacks in iron, the production of red blood cells is affected.

A person can have a low iron level because of blood loss. In women, iron and red blood cells are lost when bleeding occurs from very heavy and long periods, as well as from childbirth. Women also can lose iron and red blood cells from uterine fibroids, which can bleed slowly. Other ways iron and red blood cells can be lost include:

- Ulcers, colon polyps or colon cancer
- Regular use of aspirin and other drugs for pain
- Infections
- Severe injury
- Surgery

Eating foods low in iron also can cause IDA. Meat, poultry, fish, eggs, dairy products or iron-fortified foods are the best sources of iron found in food. Pregnancy can cause IDA if a woman doesn't consume enough iron for both her and her unborn baby. Some people have enough iron in their blood, but have problems absorbing it because of diseases, such as Crohn's disease and Celiac disease or drugs they are taking [8].

3.b Vitamins deficiency anaemia

Vitamin B12 is a product of an enzyme secreted in stomach. Deficiency of Vitamin B12 and folic acid results in megaloblastic anaemia. It is characterized by presence of large blood cell precursor in bone marrow and therefore called as maturation factor. Some people cannot produce enough Vitamin B12 and consequently could not produce enough red blood cells and causes nerve damage.This type of anaemia occurs most often in people whose bodies are not able to absorb vitamin B12 from food because of an autoimmune disorder. It also can happen because of intestinal problems [9].

Vitamin B12 is found in foods that come from animals and cereals. Folic acid supplements (pills) can treat this type of anaemia. But, folic acid cannot treat nerve damage caused by a lack of vitamin B12. Not getting enough vitamin B12 can cause numbness in legs and feet, problem in walking, memory loss, and problem in eye sight [9].

3.c Folate deficiency anaemia

This type of anaemia can occur if the enough folate is not consumed or having problems in absorbing vitamins. It also may occur during the third trimester of pregnancy, when the body needs extra folate. Folate is a Vitamin B found in foods

such as leafy green vegetables, fruits, and dried beans and peas, fortified breads, pastas and cereals [9].

4. Anaemia caused by kidney dysfunction

Some diseases can hurt the body's ability to make red blood cells. For example, anaemia is common in people with kidney disease. Their kidneys can't make enough of the hormones that signal the body to make red blood cells. Plus, iron is lost in dialysis (what some people with kidney disease must have to take out waste from the blood).

Erythropoietin is a factor produced by the kidney that stimulates erythrocyte production and various other cells to produce haemopoietic growth factor like colony stimulating factors which regulate the production of platelets, leucocytes and other blood cell types. Colony-stimulating factors (CSFs) stimulate blood cell progenitor cells to proliferate and differentiate[13].

5. Anaemias caused by inherited blood disease

Anaemia also caused by untreated blood disorders. Here are some types,

5.a Sickle cell anaemia

The red blood cells of people with sickle cell disease are hard and have a curved edge. These cells can get stuck in the small blood vessels, blocking the flow of blood to the organs and limbs. The body destroys sickle red cells quickly. But, it can't make new red blood cells fast enough [8].

5.b Aplastic anaemia

This is a rare blood disorder in which the body stops making enough new blood cells. All blood cells like red blood cells, white blood cells and platelets are

affected. Low levels of red blood cells leads to anaemia. With low levels of white blood cells, the body is less able to fight infections. With too few platelets, the blood can't clot normally. This can be caused by many things:

• Cancer treatments (radiation or chemotherapy)

• Exposure to toxic chemicals (like those used in some insecticides, paint and household cleaners)

• Some drugs (like those that treat rheumatoid arthritis)

• Autoimmune diseases (like lupus)

• Viral infections

• Family diseases passed on by genes[8].

5.c Thalassemia

People with thalassemia make less haemoglobin and fewer red blood cells than normal. This leads to mild or severe anaemia. One severe form of this condition is Cooley's anaemia[11].But the major cause is bone marrow depression, the destruction of red blood cells and loss of blood though internal bleeding or abnormal menstrual discharge[8].

HAEMOLYTIC ANAEMIA

Hemolytic anaemia is the destruction of red blood cells from the circulation before their normal lifespan of 120 days[1].

There are two mechanisms of hemolysis. Intravascular hemolysis is the destruction of red blood cells in the circulation with the release of cell contents into

the plasma. Mechanical trauma from a damaged endothelium,complement fixation and activation on the cell surface and infectious agents may cause direct membrane degradation and cell destruction.

The more common extravascular hemolysisis the removal and destruction of red blood cells with membrane alterations by the macrophages of the spleen and liver. Circulating blood is filtered continuously through thin walled splenic cords into the splenic sinusoids.

Role of G6PD in red blood cell

Reduced activity of Glucose 6-phosphate dehydrogenase impairs with the cell to form NADPH that is essential for the maintenance of reduced glutathione level. This results in decrease in the cellular detoxification of free radicals and peroxides formed within the cell and its also helps to maintain the sulphydryl group. Oxidation of this sulphydryl groups leads to the formation of **denatured proteins** that form insoluble masses (Heinz bodies) that attached to red blood cell membrane. Additional oxidation of membrane proteins causes the red blood cells to be rigid and they are removed from the circulation by macrophages in the spleen and liver.

G6PD deficiency is the major cause for hemolytic anaemia and it is due to inability to detoxify oxidizing agents. Oxidant drugs like sulphamethoxazole, chloramphenicol and antipyretics like acetaminophen cause hemolytic anaemia. In inflammation associated with the infection there is a generation of free radicals in macrophages, and cause oxiadative damage[10].

Pyruvate kinase deficiency

Pyruvate kinase deficiency is the second most common cause after G6PD deficiency of enzyme deficiency related to hemolytic anaemia.

The normal, mature erythrocyte lacks mitochondria therefore it completely dependent on glycolysis for the production of ATP. This high energy compound is required to meet the metabolic needs of the red blood cell and also necessary for the maintenance of the biconcave flexible shape of the red blood cells, which allows the cells to squeeze through narrow capillaries.

The reduced rate of glycolysis leads to decreased ATP production. It further leads to alterations in the red blood cell membrane and leads to changes in the shape of the red blood cell and cause phagocytosis by the cells of the reticulo endothelial system, particularly macrophages of the spleen. The premature death and lysis of red blood cells results in haemolytic anemia. Pyruvate kinase deficiency is restricted to erythrocytes and produce mild to severe chronic anaemia. The severity of the disease is depends upon the degree of the enzyme deficiency[10].

OXIDATIVE STRESS IN RBC

The RBC's are the first cell to be affected by oxidative stress. As they pass through lungs they carry free radicals along with oxygen. The RBC's contain high level of cytoplasmic antioxidant both enzymatic and non enzymaticin order to protect the RBC from deleterious effect of oxidative stress.The α-tocopherol, ascorbic acid and reduced glutathione level are the major endogenous antioxidants.

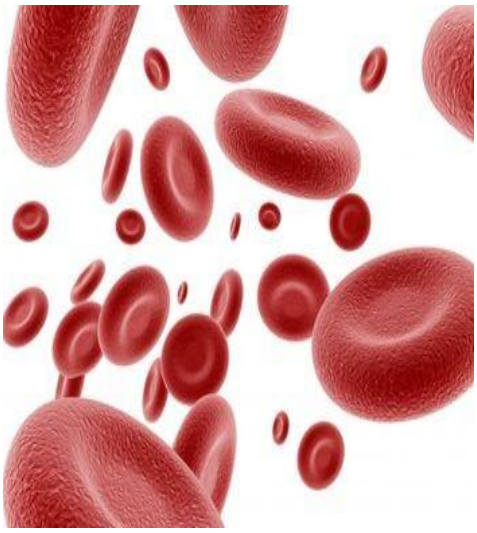
αtocopherol protects he PUFA in the RBC membrane against lipid peroxidation. Ascorbic acid, a potent cellular antioxidant protects the cell membrane from oxidative damage and also regenerate oxidized α-tocopherol. Reduced glutathione in erythrocytes maintain haemoglobin in its native form.

ROS induced cell membrane damage allows the GSH to pass through the membrane causing depletion of GSH in the cytoplasm of RBC. As the erythrocytes have no nucleus and ribosome and cannot regenerate GSH and enzyme thus becoming vulnerable to oxidative damage [10].

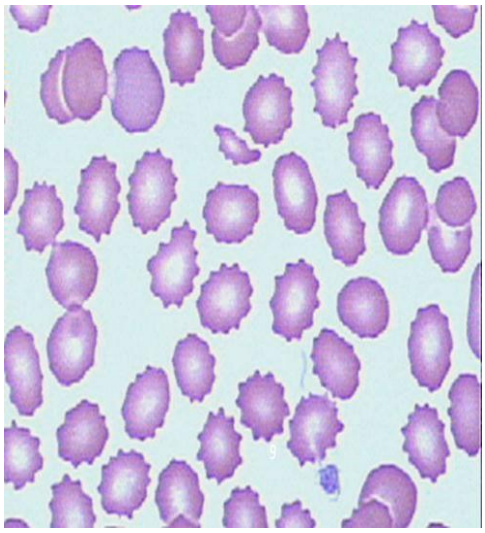
Glutathione maintains the reduced state of sulphydryl groups in proteins including haemoglobin. Oxidation of sulphydryl groups leads to the formation of denatured proteins that forms insoluble masses called as **'Heinz bodies'** that attaches to cell membrane. Oxidation of the cell membrane causes rigidity of the RBC which are removed from the surface by macrophage in the spleen and liver. Higher degree of oxidation ultimately results in haemolysis of RBC's. Reticulocytes are visualized in the peripheral blood as polychromatophilic red cell which is the cause for macrocytosis[11].

Figure 1shows the structural changes in the RBC due to oxidative haemolysis

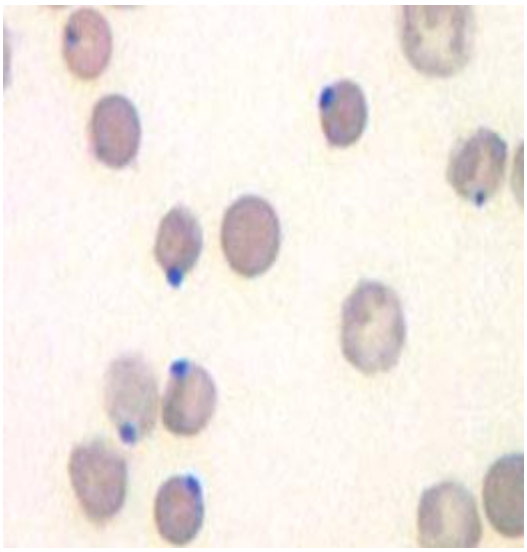
1. Normal red blood cell without oxidative stress.
2. Irregularly contracted and crenated cells- due to altered cell membrane integrity[12].
3. Presence of Heinz bodies- due to denatured haemoglobin[11].



Normal RBC's without oxidative stress



RBC's with crenated edges



Presence of Heinz bodies

SIGNS AND SYMPTOMS OF ANAEMIA[7]

The symptoms of anaemia are fatigue, weakness, dizziness, headache increased thirst numbness or coldness in hands and feet, low body temperature, pale skin, rapid or irregular heartbeat, shortness of breath, chest pain and irritability.

RISK FACTORS[7]

Infants and children, premenopausal women, elder persons, alcoholism, iron-poor diets, chronic or critical illnesses, excessive exercise and pregnancy.

REATMENT OF ANAEMIA

ANTI ANAEMIC DRUGS

Many treatment options are available for anaemia, but the primary choice is antianaemic drugs. Which treatments are used depends upon the type, location and grade of the anaemia as well as the person's health and wishes. The treatment intent may be curative or not curative[13].

Preparationof Iron

Oral preparations

- Ferrous sulphate
- Ferrous gluconate
- Ferrous fumarate
- Carbonyl ferric hydroxide
- Carbonyl iron

Parenteral preparations

- Iron sorbitol citric acid complex
- Iron dextran
- Ferrous sucrose and ferric carboxymaltose[14].

Ferrous Sulphate

Ferrous sulphate contains 20%(hydrated salt) and 32%(dried salt) elemental iron. It is the oldest and cheapest iron preparation.

Indications: Iron deficiency anaemia, blood loss related to pregnancy or GI bleeding (NSAIDs), hookworm infestation, or excess coffee.

Iron Dextran

Iron dextran is one of the parenteral preparation of iron supplement. It can be administered by intravenously or intramuscularly.

Indications

- Patients with documented iron deficiency in whom oral administration is unsatisfactory or impossible (e.g. malabsorption syndrome, prolonged salicylate therapy, dialysis patients).
- Seldom used now (newer orally effective agents including iron sucrose & ferric gluconate complex are most commonly used)[8].

Vitamin B12

Vitamin B12 is a cobalt containing compound and present in food of animal origin, such as meat, liver, egg, fish, etc.

Indications

- Used to treat or prevent deficiency of Vit B12.
- The most common causes of Vit B12 deficiency are:
- Pernicious anaemia (results from defective secretion of intrinsic factor by the gastric mucosal cells)

- Fish tapeworm infection
- Partial or total gastrectomy
- Various intestinal disorders that impair absorption of Vit B12[9].

Folic acid

Folic acid is a combination of glutamic acid, para-aminobenzoic acid and pteridine nucleus. It is found in fresh leafy vegetables, liver, yeast, fruits etc.

Indications

- Treatment of megaloblastic anaemias due to a deficiency of folic acid as may be seen in tropical or non-tropical sprue, in anaemias of nutritional origin, pregnancy, infancy, or childhood
- A reduced form of folic acid known as citrovorum factor (or leucovorin**)** is given to replenish endogenous folic acid in patients on methotrexate (which inhibits dihydrofolate reductase). Citrovorin (leucovorin) is better absorbed compared to folic acid[9].

Erythropoietin (Epoetin alpha)

Indications

- Chronic renal failure patients
- Zidovudine-treated HIV-infected patients
- Cancer patients on chemotherapy

- Reduction of allogeneic blood transfusion in surgery patients. Erythropoietin deficiency can result from compromised renal function (its primary site of production). Erythropoietin deficiency results in a normocytic anaemia[13][14].

Treatment & side effects

Based on the type of the anaemia, the treatment varies, those are Iron, Folic acid, Vitamin B12 supplements and erythropoietin are most commonly prescribed. Although these drugs have remarkable treatment, many of them have high incidence of adverse effects like nausea, abdominal pain, constipation or diarrhoea, fever, urticaria, bronchospasm, allergic sensitization even circulatory collapse and death also reported in overdose of iron (1-2g)[14].

Traditional treatment

Indigenous systems of medicine like Siddha, Ayurveda and Unani are mainly use medicinal plants for treatment of various ailments of the human beings and animals. With the development of these systems, the herbal plants are being sought after, both by clinicians and patients in search for cure of diseases. Herbal medicine is a form of complementary and alternative medicine and is becoming increasingly popular in both developing and developed countries[15].

WHO has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population.In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has encouraged the rational use of traditional plant based medicines by member states and has developed technical guidelines for the assessment of herbal medicines[16].

The herbal drugs have been used throughout the world and have raised greater attention in recent times, because of their diverse nature of curing diseases, safety and well tolerated remedies compare to the conventional medicines. Moreover the herbs with natural combinations of constituents as a whole are naturally occurring remedies which have proved to be more effective and safer than conventional medicines[17].

Herbs that are used as anti-anaemic agents with lesser side effects are *Amaranthus paniculata, Amaranthus spinosus, Basella rubra, Corriander sativum, Digella muricata, Eclipta alba, Hibiscus cannabis, Mentha piperita, Moringa olifera, Murraya koenigii, Solanum nigrum, Trigonella foeumgraecum*[4][18].

Phyllanthus emblica **Linn.,**belonging to Phyllanthaceae well known as Nellikai in Tamil is an annual herbaceous plant. Mainly grown in India is used as a traditional healer of many ailment and it was revealed by various research works and has its own importance in Ayurveda to treat fever, inflammation peptic ulcer. Analgesic, digestive, hair tonic diuretic, anti tussive, cardio protective, chemo preventive, free radical scavenging activity, anti mutagenic, cancer, anti diabetic[19][20].

Since no work done on Streptozocin induced anaemia in rats with the fruits of *Phyllanthus emblica*Linn.,therefore it is carried out for the beneficial effects of human use.

Aim and Objective

2 AIM AND OBJECTIVE

1. Extraction of active constituents present in the fruits of *Phyllanthus emblica* Linn., using distilled water : ethanol (70:30) by cold maceration method.

2. Evaluation of *in vivo* anti-anaemic activity on Streptozotocin induced adult wistar rats.

3. Evaluation of haematological parameters such as

    - RBC count
    - Haemoglobin level
    - Packed cell volume
    - Mean cell volume
    - Mean cell haemoglobin
    - Mean cell haemoglobin concentration
    - Red cell distribution width

4. Red Blood Cell morphological studies.

    - Variation in the size of RBC
    - Variation in the shape of RBC

Review of Literature

3 REVIEW OF LITERATURE

Antidiarrheal and Antispasmolytic activities

Malik Hassan Mehmood *et al.,* 2011 was evaluated about the crude extract of *Phyllanthus emblica* for its constituents like alkaloids, tannins, terpenes, flavonoids, sterols and coumarins and it causes the inhibition of castor oil-induced diarrhea and intestinal fluid accumulation in mice at the dose of 500–700mg/kg. These results indicate that the *Phyllanthus emblica* fruit extract possessed antidiarrheal and spasmolytic activities mediated possibly through dual blockade of muscarinic receptors and Ca^{2+} channels [21].

Antioxidant activity in Pregnancy

Ragini Sharma *et al.,* 2015 was stated that *Emblica officinalis* (Amla) is having good antioxidant effect and have lactating properties so it is beneficial to improve health of young ones when their mothers are fed with this plant product during pregnancy and lactation. During pregnancy there are more possibilities to generate free radicals which cause various metabolic disorders. To avoid side effects of free radicals during pregnancy women should take products with plenty of antioxidants and little deleterious impact. Amla fruit is versatile product with pharmaceutical and therapeutic properties. This study proved that *Emblica officinalis* is beneficial during pregnancy and lactation [22].

Antimutagenicity

D. Madhavi *et al.,* 2007 was investigated about the protective effect of *Phyllanthus emblica* against clastogenicity induced by lead nitrate on the incidence of sperm head abnormalities in the germ cells of mice. At higher

concentration of lead, a significant increase in the percentage of sperm head abnormalities was noted. But when the animals are treated with Phyllanthus fruit extract there was a reduction in the frequency of sperm head abnormalities is observed. It can be suggested from this study *Phyllanthus emblica* plays a key role in inhibition of heavy metal mutagenesis in mammals [23].

Antihyperlipidemia

B Antony *et al.,* 2012 was labelled that two doses of AmlamaxTM (purified, standardized, dried extract of amla) were evaluated 500 and 1000mg per day for 6 months. Blood samples were collected at the 3rd and 6th months showed reduction in total LDL cholesterols and enhancement of beneficial HDL cholesterol. In addition, blood CRP levels, a marker for inflammation, were also significantly reduced. Since hyperlipidemia is the two major component of cardiovascular diseases, the present study must be considered encouraging and indicate the potential of AmlamaxTM in the management of heart diseases [24].

Anti-inflammatory activity

Dang GK *et al.,* 2011 was studied about two models of acute inflammation, namely carrageenan induced rat paw oedema and acetic acid induced peritonitis in mice. In the model of carrageenan induced paw oedema *Phyllanthus emblica* showed a trend to reduce the oedema. Whereas in a model of acetic acid induced peritonitis *Phyllanthus emblica* showed a significant decrease in the protein content of the peritoneal exudates compared with the disease control group [25].

Anti-tumour activity

Jose JK *et al.*, 2001 was evaluated that aqueous extract of *Emblica officinalis* (E.O) was found to be reduce solid tumours in mice induced by DLA cells. Animals treated with E.O extract increases the life span of tumour bearing animals (20%). E.O extract was found to inhibit cell cycle regulating enzymes cdc-25 phosphatase in a dose dependent manner. The results suggest that anti-tumour activity of E.O extract may partially be due to its interaction with cell cycle regulation [26].

Memory enhancing activity

Vasudevan M *et al.*, 2007 was studied about memory enhancing activity of Anwala churna (Ayurvedic preparation of *Emblica officinalis*) on memory, and brain cholinesterase activity in mice. Diazepam and scopolamine are used to age-induced amnesia. Anwala churna was administered for fifteen days to different groups of young and aged mice. Elevated plus maze and passive avoidance apparatus served as the behavioral models for testing memory. Anwala churna produced a dose-dependent improvement in memory scores of young and aged mice. Furthermore, it reversed the amnesia induced by scopolamine and diazepam. Anwala churna may proved to be a useful remedy for the management of Alzheimer's disease on account of its multifarious beneficial effects such as, memory improving property and anticholinesterase activity [27].

Ophthalmic disorders

Biswas NR *et al.,* 2011 was conducted an open prospective multi centre clinical trial in patients suffering from various ophthalmic disorders like conjunctivitis, conjunctival xerosis (dry eye), acute dacryocystitis, degenerative conditions (pterygium or pinguecula) and postoperative cataract patients with a herbal eye drop preparation (Ophthalcare). Ophthal care include *Carum copticum, Terminalia belirica, Emblica officinalis, Curcuma longa, Ocimum sanctum, Cinnamomum camphora, Rosa damascena* and *Meldespum apum*. An improvement was observed with the treatment of the herbal eye drop treatment in most cases. There were no side effects observed during the course of the study and the eye drop was well tolerated by the patients [28].

Antidiabetic activity

Mai A Elobeid *et al.,* 2015 was studied about the anti hyperglycemic effect of the aqueous fruit extract of amla. Diabetes was induced by streptozotocin injection at the dose of 45mg/kg. Untreated diabetic control (group 1) received 2 % gum acacia as vehicle; groups 2 and 3 were diabetic rats administered the fruit extract in 400 and 200 mg/kg doses, respectively; while group 4 (diabetic rats) received metformin (600 mg/kg) as reference drug. The parameters assessed weekly were body weight, as well as fasting blood glucose, cholesterol and triglyceride levels in venous blood. Both plant extract-treated groups showed significant reduction in blood glucose levels, cholesterol and triglyceride levels, respectively, similar to the metformin treated group. This study also stated that the anti-diabetic activity of the aqueous extract of *E. officianalis* showed a better potential than metformin [29], [30].

Immunomodulatory effect

Singh MK *et al.,* 2013 was studied the protective effect of amla against thymocytes in mice. Mice were exposed to arsenic induced oxidative stress and apoptosis. The antioxidant enzyme assays were carried out using spectrophotometer and generation of ROS, apoptotic parameters, change in cell cycle were carried out using flow cytometer following the standard protocols. Arsenic exposure to mice caused a significant increase in the lipid peroxidation, ROS production and decreased cell viability, levels of reduced glutathione, the activity of superoxide dismutase, catalase, cytochrome c oxidase and mitochondrial membrane potential in the thymus as compared to controls. Co-treatment with arsenic and amla decreased the levels of cell viability, levels of antioxidant enzymes, cytochrome c oxidase and mitochondrial membrane potential as compared to mice treated with arsenic alone. The results was proved that arsenic induced oxidative stress and apoptosis significantly protected by co-treatment with amla that could be due to its strong antioxidant potential [31].

Skin disorder

- **Vitiligo**

Colucci R et al 2016 was studied about the repigmenting effects of *Phyllanthus emblica* (P.E) in vitiligo treatment. Sixty-five human subjects were treated with one tablet of an oral supplement containing *Phyllanthus emblica* After a 6 months follow-up, a significantly higher number of patients in group A had a mild repigmentation on the head/neck regions and on the trunk. This result reported that the supplement with *Phyllanthus emblica* increases the effectiveness in patients with vitiligo [32].

- **Melasma**

Costa A *et al.,* 2010 was studied about the clinical efficacy and safety of the *Phyllanthus emblica* compared to Hydroquinone 2%, in the treatment of melasma. 50 volunteers (89%), 23 in Group A and 27 in Group B was concluded the study. Two volunteers in Group A and seven volunteers in Group B had mild skin adverse events. Depigmentation was observed with the use of *Phyllanthus emblica* it is a safe and efficient alternative for the treatment of melasma [33].

Antiarthritic activity

Venil N *et al.,* 2007 was performed an *in vitro* study to measure the chondroprotective effect of *P. emblica* ('Amalaki') fruits by cartilage degradation. The *in vitro model* was setup with explant cultures of articular knee cartilage from osteoarthritis patients. Cartilage damage was assayed by measuring glycosaminoglycan release from explants treated with *P. emblica* fruit powders. Aqueous extracts of fruits of *Phyllanthus emblica* powders significantly inhibited the activities of hyaluronidase and collagenase in *in vitro study*. This study is performed to identify the chondro protective activity of *P. emblica* fruits and it was found to be possess antiarthritic activity [34].

Anticoagulant

Rao TP *et al.,* 2013 was reported that the amla fruit extract potentially reduce the lipo polysaccharide (LPS)-induced tissue factor expression and von Wille brand factor release in human umbilical vein endothelial cells (HUVEC). In addition, the *in vivo* anti-inflammatory effects were evaluated in a LPS-

induced endotoxaemia rat model. Oral administration of the amla fruit extract (50mg/kg body weight) significantly decreased the concentrations of pro-inflammatory cytokines, TNF-α and IL-6 in serum. These results suggested that amla fruit extract may be an effective anticoagulant and anti-inflammatory agent [35].

Prokinetic, laxative and spasmodic activities

Mehmood MH *et al.,* 2013 was evaluated crude extract of the dried fruits of *Phyllanthus emblica* (P.E.) and its fractions were tested positive for the prokinetic and laxative activities in mice. In isolated guinea-pig ileum and rabbit jejunum, the crude extract and its aqueous fraction (P.E.) caused concentration-dependent and partially atropine-sensitive stimulatory effects followed by relaxation at higher tested concentrations, being more efficacious in guinea pig, while more potent in rabbit tissues. This study showed that the *Phyllanthus emblica* possessesd prokinetic and laxative activities in mice along with spasmodic effect in the isolated tissues of guinea pig and rabbit, mediated partially through activation of muscarinic receptors. This study also provides a rationale for the medicinal use of *Phyllanthus emblica* fruits in indigestion and constipation [36].

Radioprotective effect

Jindal A *et al.,* 2009 was studied about the radioprotective effect *of Emblica officinalis extract* (EOE) in mice. Swiss albino mice were exposed to gamma rays to produce toxicity. Then EOE is orally administered at the dose of 100 mg/kg body weight once daily for 7 consecutive days. A specimen of small intestine (jejunum) was removed from the mice and studied at different autopsy

intervals from 12 hours to 30 days. The animals receiving EOE prior to irradiation had a higher number of crypt cells and mitotic figures when compared with non-drug-treated control at all the autopsy intervals. Irradiation of animals resulted in a dose-dependent elevation in lipid peroxidation and a reduction in glutathione as well as catalase concentration in the intestine at 1 hour post-irradiation. In contrast, EOE treatment before irradiation caused a significant depletion in lipid peroxidation and elevation in glutathione and catalase levels and showed significant radioprotective effect[37].

Wound Healing Activity

Hema Sharma Datta *et al.*, 2011 was evaluated the wound healing activity in rats. In this study the parameters studied are including wound contraction, collagen content and skin breaking strength which in turn an indicative of the tissue cell regeneration capacity, collagenation capacity and mechanical strength of skin. The group treated with the formulations containing *Phyllanthus emblica* showed significantly better wound contraction, higher collagen content and better skin breaking strength when compared to control group. *Phyllanthus emblica* was found to possess wound healing activity [38].

Antisnake venom activity

Alam MI *et al.*, 2003 evaluate the antisnake venom activity of methanolic root extracts of *Vitex negundo* Linn and *Emblica officinalis*. E. officinalis extracts significantly antagonized the Vipera russellii and Naja kaouthia venom induced lethal activity in *in vitro* and *in vivo* studies. V. russellii venom-induced haemorrhage, coagulant, defibrinogenating and

inflammatory activity was significantly neutralized by both plant extracts. No precipitating bands were observed between the plant extract and snake venom. This study confirmed that methanolic root extracts of *Emblica officinalis* possessd potent snake venom neutralizing capacity [39].

Anti epileptic

Golechha M *et al.,* 2011 was evaluated the anti epileptic activity of hydro alcoholic extract of *Emblica officinalis* fruit (HAEEO) in rats. Seizure was induced by the administration of kainic acid (KA) and observed for behavioral changes, incidence, and latency of convulsions over 4 hours. The rats were thereafter sacrificed for estimation of oxidative stress parameters such as thiobarbituric acid-reactive substances (TBARs) and glutathione (GSH). The pro inflammatory cytokine tumor necrosis factor alpha (TNF-α) was also determined in the rat brain. Pre treatment with HAEEO significantly increases the latency of seizure TNF-α level in the brain and prevented the increase in TBARS levels and ameliorated the fall in GSH. These reports stated that *Emblica officinalis* fruit possess efficient anti epileptic activity [40].

Arsenic toxicity in water

Sayed S *et al.,* 2015 was conducted an experiment to evaluate the protective effects of *Phyllanthus emblica* leaf extract (PLE) on arsenic-mediated toxicity in mice. Male Swiss albino mice were divided into three different groups (n=6/group). 'Control' mice received arsenic free water together with normal feed. Mice in the remaining two groups designated 'SA' and 'SA+PLE' were exposed to sodium arsenite (SA) through drinking water in addition to receiving normal feed and PLE-supplemented feed, respectively. The weight

gain of SA-exposed mice was decreased compared with the controls. A secondary effect of arsenic was enlargement of the liver, kidney and spleen was observed in SA-group mice. In PLE supplemented mice the enlargement of the organs was minimized and the deposition of arsenic was not significantly reduced. PLE may not block arsenic deposition in tissue directly but rather may play a protective role to reduce arsenic-induced toxicity [41].

PLANT PROFILE

Figure 2 Fruits of *Phyllanthus emblica* Linn.,

PLANT PROFILE

Botanical name : *Phyllanthus emblica* L.

Synonym : *P. Mairei, Emblica officinalis* Gaertn., *P. Taxifolius* D. Don

Family : Phyllanthaceae [42]

Toxonomical classification [19], [44]

Kingdom	:	Plantae
Order	:	Malpighiales
Family	:	Phyllanthaceae
Genus	:	Phyllanthus
Species	:	*P. emblica*
Botanical name	:	*Phyllanthus emblica* L.
Synonyms	:	*P. Mairei*, *Emblica officinalis* Gaertn., *P. taxifolius* D. Don

Vernacular names [43], [19]

Sanskrit	:	Dhatriphala, Amla, Amaliki, Amalakan
Tamil	:	Nellikai
Hindi	:	Amla
English	:	Emblica myroblan
Telugu	:	Rasi usiri
Malayalam	:	Nelli
Oriya	:	Anlaa
Punjabi	:	Aula

Description

Phyllanthus emblica is small to medium sized, reaching 8 to 18 m in height, with a crooked trunk and spreading branches. The branch lets are glabrous or finely pubescent, 10-20 cm long, usually deciduous. The leaves are simple, sub sessile and closely set along branch lets, light green, resembling pinnate leaves. The flowers are greenish-yellow. The fruit is nearly spherical, light greenish yellow, quite smooth and hard on appearance, with 6 vertical stripes or furrows. Ripening in autumn, the berries are harvested by hand after climbing to upper branches bearing the fruits. The taste of Indian gooseberry is sour, bitter and astringent, and is quite fibrous [44].

Habitat and Cultivation

It is a potential crop which grows in marginal soils and various kinds of degraded lands such as salt-affected soils, dry and semi-dry regions. Found wild and cultivated in all parts of India, especially the south, to an altitude of 1500 m. Although it is native to India, also grows in tropical and subtropical regions of Pakistan, Uzbekistan, Sri Lanka, South East Asia, China, Malaysia, Indonesia, Peninsula, Burma and Ceylon [45][46].

Parts used

Fresh and dried fruit, seed, leaf, root, bark and flower [19].

Chemical constituents

Tannins 30%, phyllembin (2.4%), phyllemblic acid (6.3%), gallicacid (1.32%), ellagic acid in natural form and cytokine like substances identified as Zeatin, Z riboside, Z nucleotide etc. Amla is a rich natural source of vitamin C. It contains pectin & 75% moisture**.** Amla fruit ash contains chromium 2.5, zinc 4 and copper 3 ppm [19] [43].

Traditional uses [19]

- Reduces fever and cough
- Purifies the blood
- Stimulate hair growth
- Enhances the digestion
- Used in inks, shampoos and hair oils.

Medicinal uses [43] [19]

- Decoction prepared of dried fruit in new earthen vessel is used for eye wash in ophthalmic diseases.
- The exudate collected from incisions made on the fruit is applied externally on inflammation of the eye.
- Fermented liquor prepared from the fruit is used in jaundice, dyspepsia and cough.
- Paste of the fruit is a useful external application over the pubic region in irritability of the bladder.
- Fruits are used as an expectorant, an antidote to mineral poisons, particularly vermillion and sulphur.
- Fruit with seeds used for asthma, bronchitis and biliousness.
- Amla juice with turmeric powder and honey is beneficial in diabetes insipidus.
- In Persia the juice of the fruit is used as a vermifuge.

- In Tibetan medicine, the fruit have been used as anti pyretic, anti inflammatory and anti-diuretic.
- Indonesians used this fruit for the treatment of dysentery, diarrhoea, cholera and biliary disorders.
- The pulp of the fruit is smeared on the head to dispel headache and dizziness.
- In Turkistan the fresh fruit is used in inflammation of the lungs.
- Juice of the fruit along with other ingredients is used to cure cough, hiccough, asthma as well as fits and insanity.

Scope and Plan of Work

4 SCOPE AND PLAN OF WORK

- Anaemia is the blood disorder characterized by reduction in the total number of red blood cells, hemoglobin concentration and packed cell volume. In India, anaemia became the major public health challenge that attacks preschool children and women at reproductive age. In 2002-2004-DLHS (District Level Health Service) reported that, 98% of adolescent girls and 96% of pregnant women are suffered from anaemia. In 2005-2006- NFHS (National Family Health Survey) reported that 24% of men are also found to have anaemia due to low level of hemoglobin than normal level[5].
- Recent studies are shows that herbal drugs are good source of alternative therapy which has lesser side effects compared to the conventional system of medicine.
- Medicinal plants play a major role in anti-anaemic activity and their anti oxidant action is mediated through inhibition of reactive oxygen species.
- The fruits of *Phyllanthusemblica*Linn., was selected for the study of anti-anaemic activity as there are traditional claims to treat anaemia. The literature review shows that various works on the fruit. But no scientific work has been published on streptozotocin induced anaemic rats so far.
- Hence the present study attempts to study the anti-anaemic activity on the fruits of *Phyllanthus emblia* Linn.,

PLAN OF WORK

Fruits of *Phyllanthus emblica* Linn.,

Collection of plant material

Authentication and Processing of fruits

Aqueous extraction by cold maceration method

In vivo anti- anaemic activity

Hematological parameters

- RBC count
- Haemoglobin level
- Packed cell volume
- Mean cell volume
- Mean cell haemoglobin
- Mean cell haemoglobin concentration
- Red cell distribution width

RBC Morphology studies

- Variation in the size of RBC
- Variation in the shape of RBC

Materials and Methods

5MATERIALS AND METHODS

1. Collection and identification of plant material

The fruits of *Phyllanthus emblica* Linn. were collected from the garden in Namakkal district, Tamil Nadu in the month of Augest, 2015. The plant was identified and authenticated by Prof. Sasikala Ethirajulu, Botanist, Siddha Central Research Institute, Arumbakkam, Chennai-600106.

2. Preparation of plant extract

Chemicals

Analytical grade of ethanol and distilled water were bought from Microfine Chemicals, Chennai.

Maceration

The fruits were washed under the tap water, shade dried at room temperature and then subjected to size reduction to a coarse powder by using wiley mill. The powdered fruit material was placed in a stoppered container with distilled water and ethanol at the ratio of 70:30 in a room temperature for a period of at least 7 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc is pressed, and the combined liquids are clarified by filtration or decantation after standing. The aqueous extract was concentrated in a rotary evaporator. The concentrated extract was taken for the *in vivo* studies.

Table No: 1 Percentage yield from the aqueous extract of

***Phyllanthus emblica* Linn.,**

Extract	Plant material used	Weight of the plant material(g)	Yield (g)	Percentage yield(%)
Distilled water: ethanol (70:30)	Dried fruits	250	7	17.5

3. Screening of anti-anaemic activity

3.1 Experimental animals

Healthy wistar rats (150-200g) were bought from Animal Experimental Laboratory, Madras Medical College, Chennai-03. The study was approved by Institutional Animal Ethics Committee which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Approval Number:02/243 CPCSEA dated on 10.08.2015

3.2 Maintenance of animals

The animals were kept in clean and dry polypropylene cages with stainless steel top grill having facilities for pelleted food and water. The animals were maintained in a well ventilated animal house in 12 hours and 12 hours dark cycle at a temperature of $28^{o}C \pm 2^{o}C$ and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals fed with standard pellet diet and water *ad libitum*. All animal experiments were performed according to the ethical guidelines suggested by Institutional Animal Ethics Committee (IAEC). Paddy husk was used as bedding material and changed twice a week[47].

3.3 Animal model

The model used to evaluate the anti-anaemic activity was Streptozotocin[48] induced anaemia in rats[49].

Streptozotocin induced anaemia in rats

Streptozotocin was bought from Microfine Chemicals, Chennai. All the chemicals used in the study were of analytical grade.

- **Procedure**

Anaemia is induced in rats by administration of Streptozotocin for 7 days at the dose of 45mg/kg, intraperitoneally. All the animals were weighed and divided into four groups, each group containing six animals[49][29].

Table No: 2 Study Design

S. No	Group	Name of the group	Dose	No. of animals	Duration of dosage
1	I	Control	Nil	6	7 weeks
2	II	Streptozotocin	45mg/kg	6	1 week
3	III	Streptozotocin+ Vit C	150kg/kg	6	6 weeks
4	IV	Streptozotocin+ water extract	500mg/kg	6	6 weeks
5	Total			24	7 weeks

The blood samples to be collected before and after the induction of anaemia by retro orbital sinus with glass capillary tube and used for the estimation of haematological parameters[50]. Then the blood was allowed to clot for 30 minutes at room temperature. The clear serum was separated by centrifugation at 2500 rpm for 5 minutes and used for the RBC morphology studies[51].

- **Parameters evaluated**

  - **Haematological analysis**

    - Red Blood Cell Volume (RBC)
    - Haemoglobin (HB)
    - Packed Cell Volume (PCV)
    - Mean cell volume (MCV)
    - Mean cell haemoglobin (MCH)
    - Mean cell haemoglobin concentration (MCHC)
    - Red cell distribution width (RDW)

  - **RBC morphology**

    - Variation in the size of RBC
    - Variation in the shape of RBC

  - **Statistical analysis**

The results of the haematological estimations were presented as mean ± SD of six animals in each group. Total variations, present in a set of data were estimated by One Way Analysis Of Variance (ANOVA). P value of <0.05 was considered statistically significant.

A. Haematological analysis

Hematology analyzers are used widely in patient and research settings to count and characterize blood cells for disease detection and monitoring[52]. Basic analyzers return a complete blood count (CBC) with a three-part differential white blood cell (WBC) count[52]. Sophisticated analyzers measure cell morphology and can detect small cell populations to diagnose rare blood conditions[53].

Figure 3shows XP-100 hematology analyzer

Principles of measurement

Blood is sampled, diluted and moves through a tube thin enough that cells pass by one at a time. Characteristics about the cell are measured using lasers (fluorescent flow cytometry)or electrical impedence. Because not everything about the cells can be measured at the same time, blood is sampled into a number of different channels.

The three main physical technologies used in hematology analyzers are: electrical impedance, flow cytometry, and fluorescent flow cytometry. These are used in combination with chemical reagents that lyse or alter blood cells to extend the measurable parameters[52].

Electrical impedance

The traditional method for counting cells is electrical impedance, also known as the Coulter principle. It is used in almost every hematology analyzer. Whole blood is passed between two electrodes through an aperture so narrow that only one cell can pass through at a time. The impedance changes as a cell passes through. The change in impedance is proportional to cell volume, resulting in a cell count and measure of volume. Impedance analysis returns CBCs and three-part WBC differentials (granulocytes, lymphocytes, and monocytes) but cannot distinguish between the similarly sized granular leukocytes: eosinophils, basophils and neutrophils. Counting rates of up to 10,000 cells per second can be achieved and a typical impedance analysis can be carried out in less than a minute[53].

Flow cytometry

Laser flow cytometry is more expensive than impedance analysis, due to the requirement for expensive reagents, but returns detailed information about the morphology of blood cells. It is an excellent method for determining five-part WBC differentials. A single-cell stream passes through a laser beam. The absorbance is measured and the scattered light is measured at multiple angles to determine the cell's granularity, diameter and inner complexity. These are the same cell morphology characteristics that can be determined manually from a slide[53].

Fluorescent flow cytometry

Adding fluorescent reagents extends the use of flow cytometry to measure specific cell populations. Fluorescent dyes reveal the nucleus-plasma ratio of each stained cell. It is useful for the analysis of platelets, nucleated RBCs, and reticulocytes.

Abbott Sapphire, Siemens Advia 120, Beckman Coulter DxH 800, and Sysmex XE-2100 are the mostly used CBC analyzers[54].

Procedure

The caps are left on the tubes as they go through the machine. A piercer takes a sample through the rubber centre while the tube is upside down. EDTA (lavender) tubes are usually used, although citrate (blue top) tubes will also work (although the result must be corrected because of dilution)[55].

Range of tests

Every haematology analyzer returns a CBC and a three- or five-part WBC differential. However, even the simplest analyzer will return multiple parameters. The most basic analyzer from Sysmex is the XP-100. It returns 20 parameters: WBC, RBC, HBB, HCT, MCV, MCH, MCHC, PLT, NEUT %, LYM%, MXD%, RDW-SD, RDW-CV,MXD, MPV, PCT, NEUT#, LYM# , P-LCR and MXD#[52][56]

Time per analysis

Time per analysis depends on the parameters being measured. A simple CBC can typically be returned in 1 min. More sophisticated analyses can take up to 10 min.

Sample size and micro sampling

Sample size requirements are on the order of 150μL of whole blood for multiparameter analysis. Many manufacturers offer micro sampling, which is particularly helpful for pediatrics. The Horiba ABX Micros ES 60 uses 10μL of whole blood for CBC plus WBC 3-differential[52].

Accuracy, precision and linearity

The accuracy of volume measurements using electrical impedance can be greater than 1% and depends on the width of the aperture relative to the cell being measured. Some instruments use multiple-sized apertures to improve accuracy for different-sized cells. Temperature also affects accuracy. The Horiba Pentra 80 features a preheated analysis chamber to ensure consistent results[52].

Open or closed tube sampling

Closed tube sampling reduces the risk of exposure to blood. Instruments with open or closed sampling options have different stability and calibration requirements depending on the mode of operation.

Results storage and analysis

A major differentiator between analyzers is the number of results that can be stored on the system. A small benchtop analyzer might typically save 1000 patient results with histograms. The Horiba ABX Pentra DX120 SPS will store 90,000 results plus graphics[52].

B. RBC Morphology

Normal and Pathologic Red Blood Cells

The normal red blood cell is a biconcave disk, 6 to 9μm in diameter and 1.5 to 2.5 μm thick. The haemoglobin imparts a uniform pink to orange-red color to the cytoplasm that is typically without inclusions[2]. Normally all red blood cells are relatively uniform in size and shape. Numerous disease states affect the size, shape and haemoglobin content of red cells. Variation in size is referred to as "anisocytosis," and variation in shape is termed "poikilocytosis." Pathologic red cells may be larger or smaller than normal, may be abnormally shaped, or may contain inclusions[57][59].

Procedure

- 1ml of blood will be collected from rat eye by sinus orbital puncture and centrifuged at 2000rpm for 5mins and the plasma was discarded.
- The packed cells are reconstituted as 10% v/v suspension with 0.9% normal saline[57].
- A Drop of this suspension will be put on a glass slide under a cover slip and studied under High Power Microscope at 40 X for assessment of morphological changes in the red blood cells[57].

Morphological changes in RBC's at 0^{th}day, after induction of anaemia and after 6 weeks of treatment with amla aqueous extract will be assessed using the following scoring pattern [60]

- 5-10% abnormal RBC =0
- 10-25% abnormal RBC = 1+
- 25-50% abnormal RBC = 2+
- 50-75% abnormal RBC =3+
- >75% abnormal RBC = 4+

Results

6 RESULTS

Table 3: Haematological parameters after the treatment with aqueous extract of *Phyllanthus emblica* Linn., at the end of 42days

S. NO	PARAMETERS	GROUP I	GROUP II	GROUP III	GROUP IV
1	RBC Count ($X10^{12}$/L)	8.98±0.562	7.23±0.468[a]	8.07±0.539[b]	7.96±0.396[b]
2	Haemoglobin(g/DL)	15.76±0.626	10.15±0.584[a]	14.78±0.686[b]	13.64±0.842[b]
3	Hematocrit(%)	46.85±1.247	38.13±1.437[a]	43.78±0.692[b]	41.26±0.725[b]
4	Mean Cell Volume (fL)	61.8±0.9927	49.2±1.434[a]	57.6±2.688[b]	54.2±1.003[b]
5	Mean Cell Hemoglobin(pg)	19.4±0.612	16.4±0.55[a]	18.2±0.206[b]	17.43±0.909[b]
6	Mean Cell Hemoglobin Concentration(g/DL)	37.18±1.592	31.4±0.864[a]	35.4±1.093[b]	34.2±0.887[b]
7	Red Cell Distribution Width(%)	14.4±0.493	10.3±0.917[a]	14.0±0.793[b]	13.4±0.686[b]

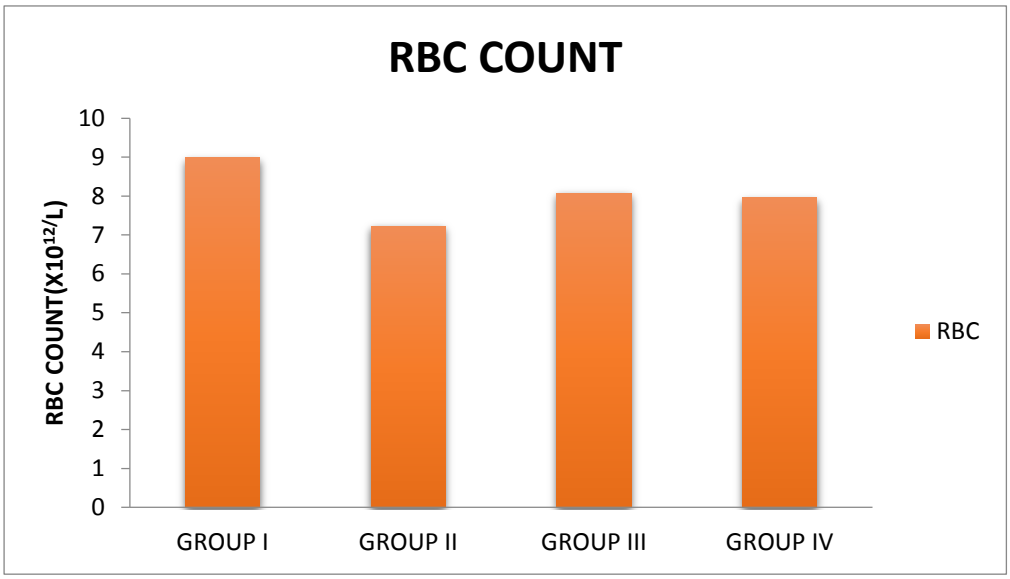
Values are expressed as mean ± SD (n=6).

'a' P<0.001 compared with control group

'b' P<0.001 compared with negative control group

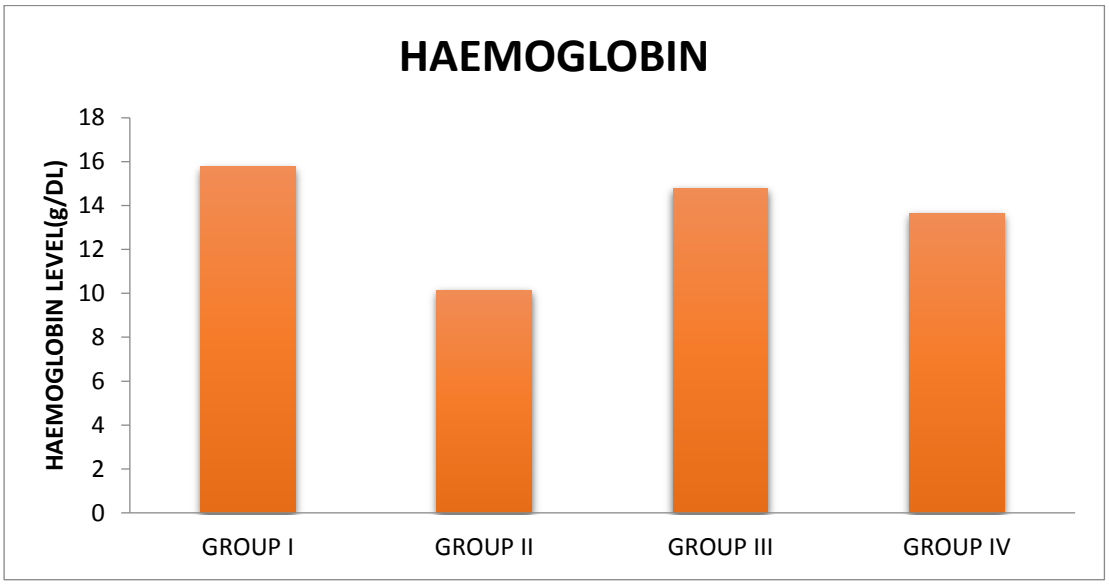
There is a decrease in RBC count, Haemoglobin level, Hematocrit, Mean cell volume, Mean cell haemoglobin, Mean cell haemoglobin concentration, Red cell distribution width after the administration of streptozotocin in negative control group. And these levels are increased with the administration of aqueous extract of *Phyllanthus emblica* Linn., treated group and the P values are also significant (P<0.001).

Figure 4: RBC count after the treatment with aqueous extract of *Phyllanthu semblica* Linn., at the end of 42 days



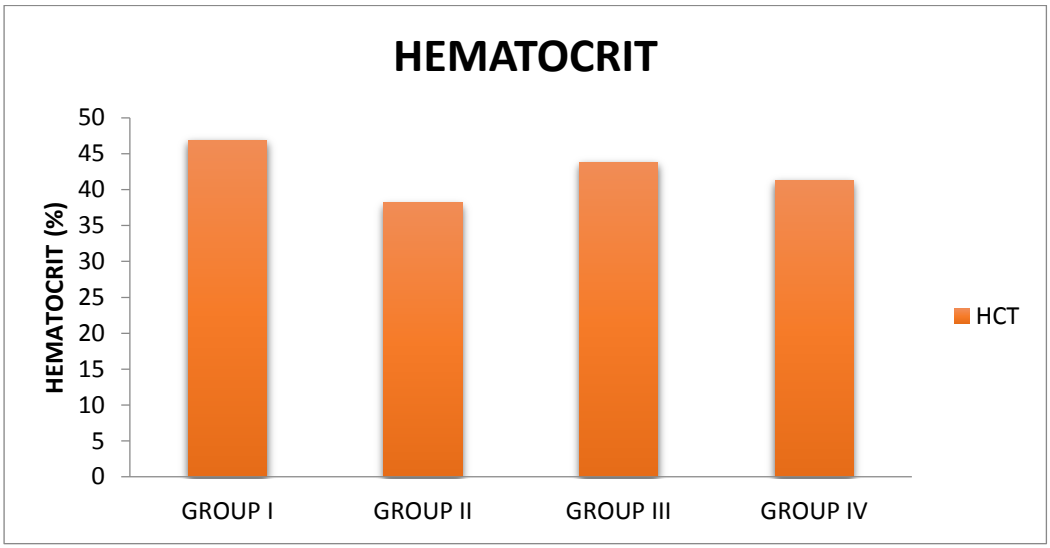
The results are tabulated in **Table 3**. Anaemic rats showed decrease in RBC count whereas the RBC count of the control rats remained the same. Administration of Vit C and aqueous extract of fruit of *Phyllanthus emblica* for 42 days significantly ($P<0.001$) increase the RBC count and brought back RBC count towards normal.

Figure 5: Haemoglobin level after the treatment with aqueous extract of *Phyllanthus emblica* Linn., at the end of 42 days



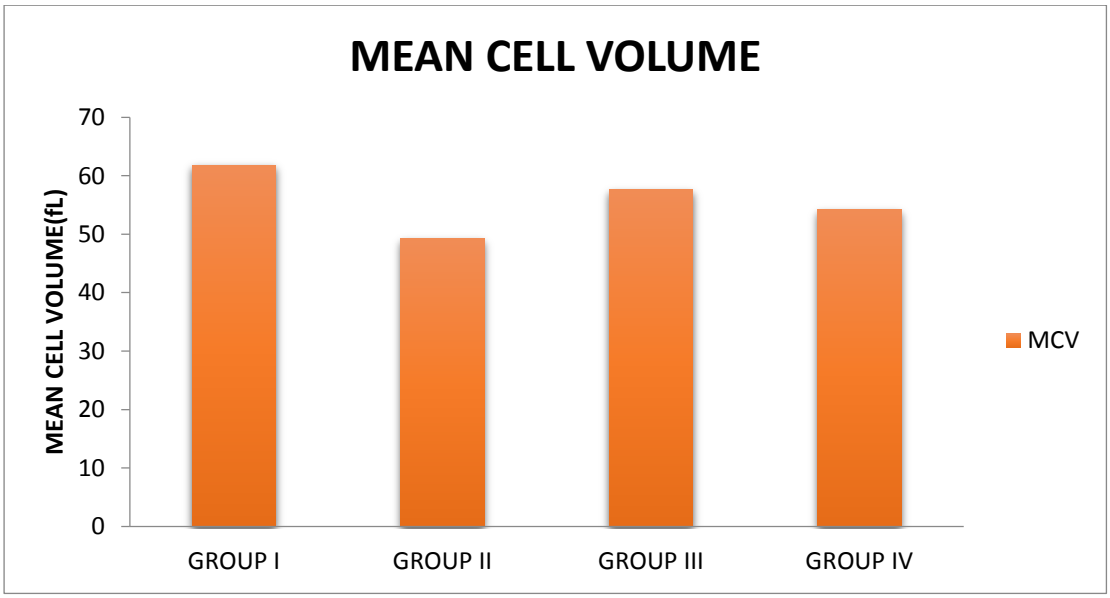
The results are tabulated in **Table 3**. Anaemic rats showed decrease in Hb level whereas the Hb level of the control rats remained the same. Administration of Vit C and aqueous extract of fruit of *Phyllanthus emblica* for 42 days significantly ($P<0.001$) increase the Hb level and brought back Hb level towards normal.

Figure 6: Hematocrit after the treatment with aqueous extract of *Phyllanthus emblica* Linn., at the end of 42 days



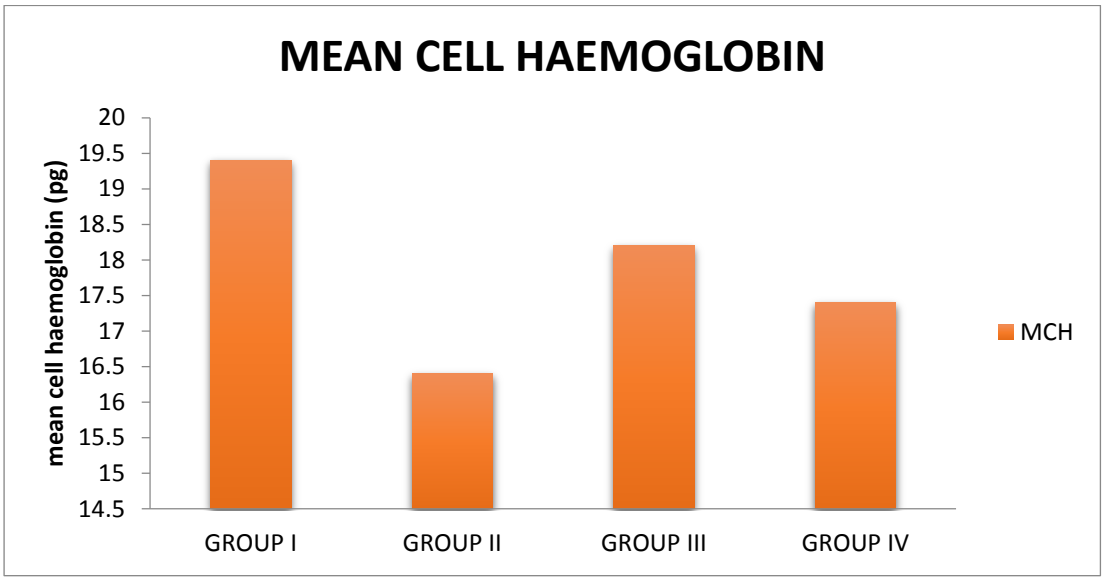
The results are tabulated in **Table 3**. Anaemic rats showed decrease in Hematocrit whereas the Hematocrit of the control rats remained the same. Administration of Vit C and aqueous extract of fruit of *Phyllanthus emblica* for 42 days significantly ($P<0.001$) increase the Hematocrit and brought back Hematocrit towards normal.

Figure 7: Mean Cell Volume after the treatment with aqueous extract of *Phyllanthus emblica* Linn,.at the end of 42 days



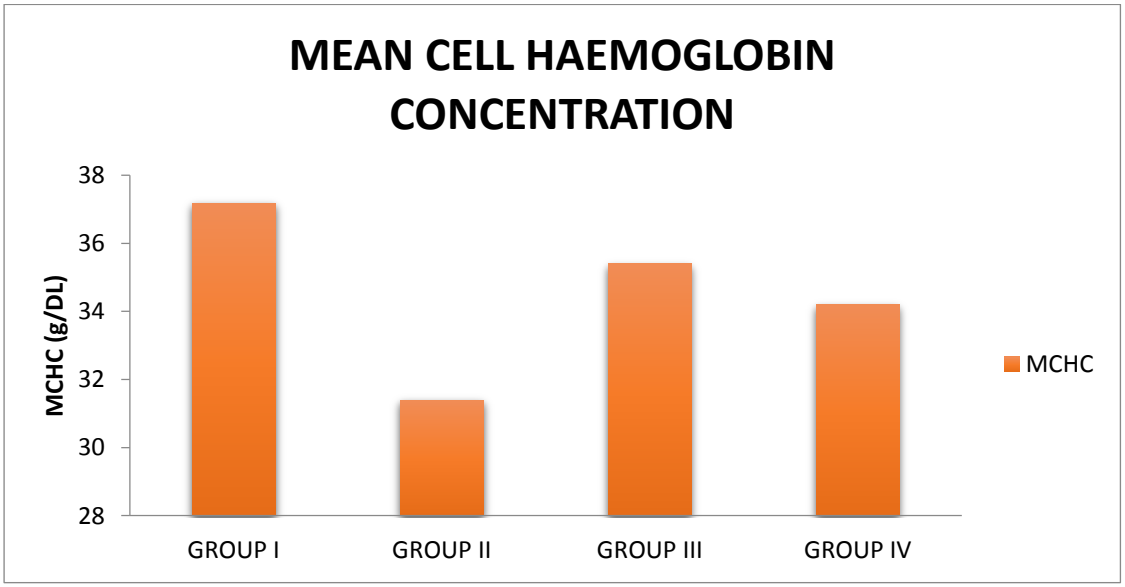
The results are tabulated in **Table 3**. Anaemic rats showed decrease in Mean Cell Volume whereas the Mean Cell Volume of the control rats remained the same. Administration of Vit C and aqueous extract of fruit of *Phyllanthu semblica* for 42 days significantly ($P<0.001$) increase the Mean Cell Volume and brought back Mean Cell Volume towards normal.

Figure 8: Mean Cell Haemoglobin after the treatment with aqueous extract of *Phyllanthu semblica* Linn., at the end of 42 days



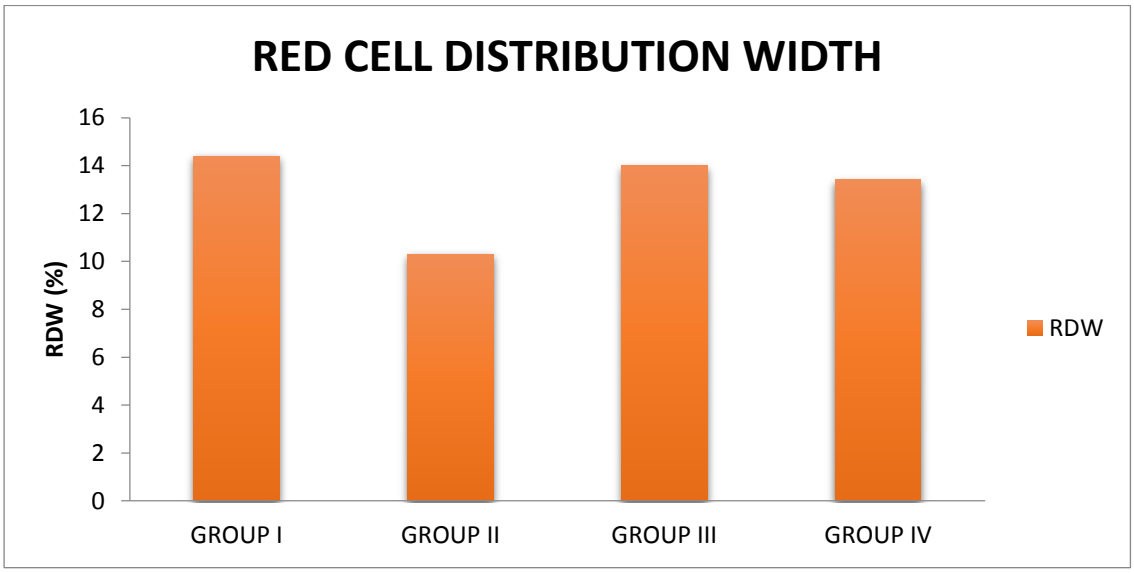
The results are tabulated in **Table 3**. Anaemic rats showed decrease in Mean Cell haemoglobin level whereas the Mean Cell haemoglobin level of the control rats remained the same. Administration of Vit C and aqueous extract of fruit of *Phyllanthus emblica* for 42 days significantly ($P<0.001$) increase the Mean Cell haemoglobin level and brought back Mean Cell haemoglobin level towards normal.

Figure 9: Mean Cell Haemoglobin Concentration after the treatment with aqueous extract of *Phyllanthus emblica* Linn., at the end of 42 days



The results are tabulated in **Table 3**. Anaemic rats showed decrease in Mean Cell Haemoglobin Concentration level whereas the Mean Cell Haemoglobin Concentration level of the control rats remained the same. Administration of Vit C and aqueous extract of fruit of *Phyllanthus emblica* for 42 days significantly ($P<0.001$) increase the Mean Cell Haemoglobin Concentration level and brought back Mean Cell Haemoglobin Concentration level towards normal.

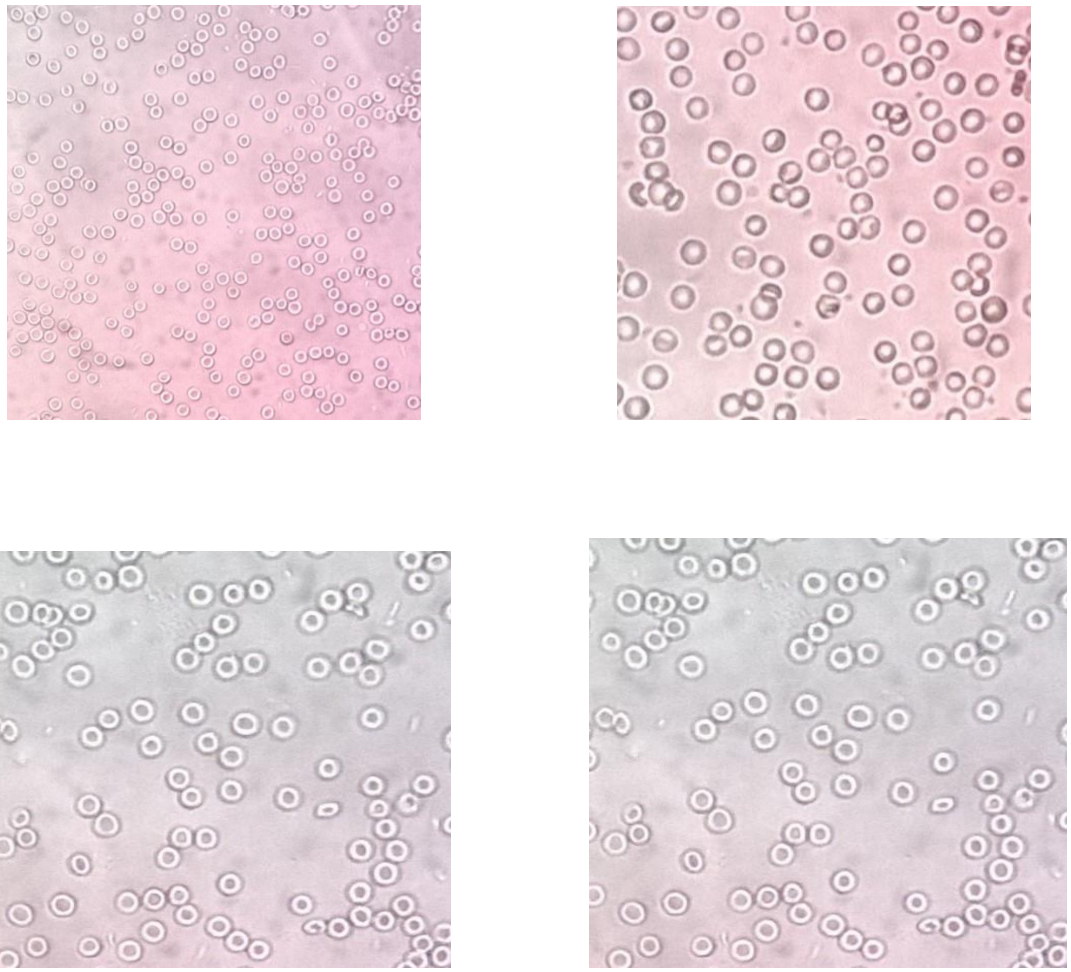
Figure 10: Red Cell Distribution Width after the treatment with aqueous extract of *Phyllanthus emblica* Linn.,at the end of 42 days



The results are tabulated in **Table 3**. Anaemic rats showed decrease in Red Cell Distribution Width whereas the Red Cell Distribution Width of the control rats remained the same. Administration of Vit C and aqueous extract of fruit of *Phyllanthus emblica* for 42 days significantly ($P<0.001$) increase the Red Cell Distribution Width and brought back Red Cell Distribution Width towards normal.

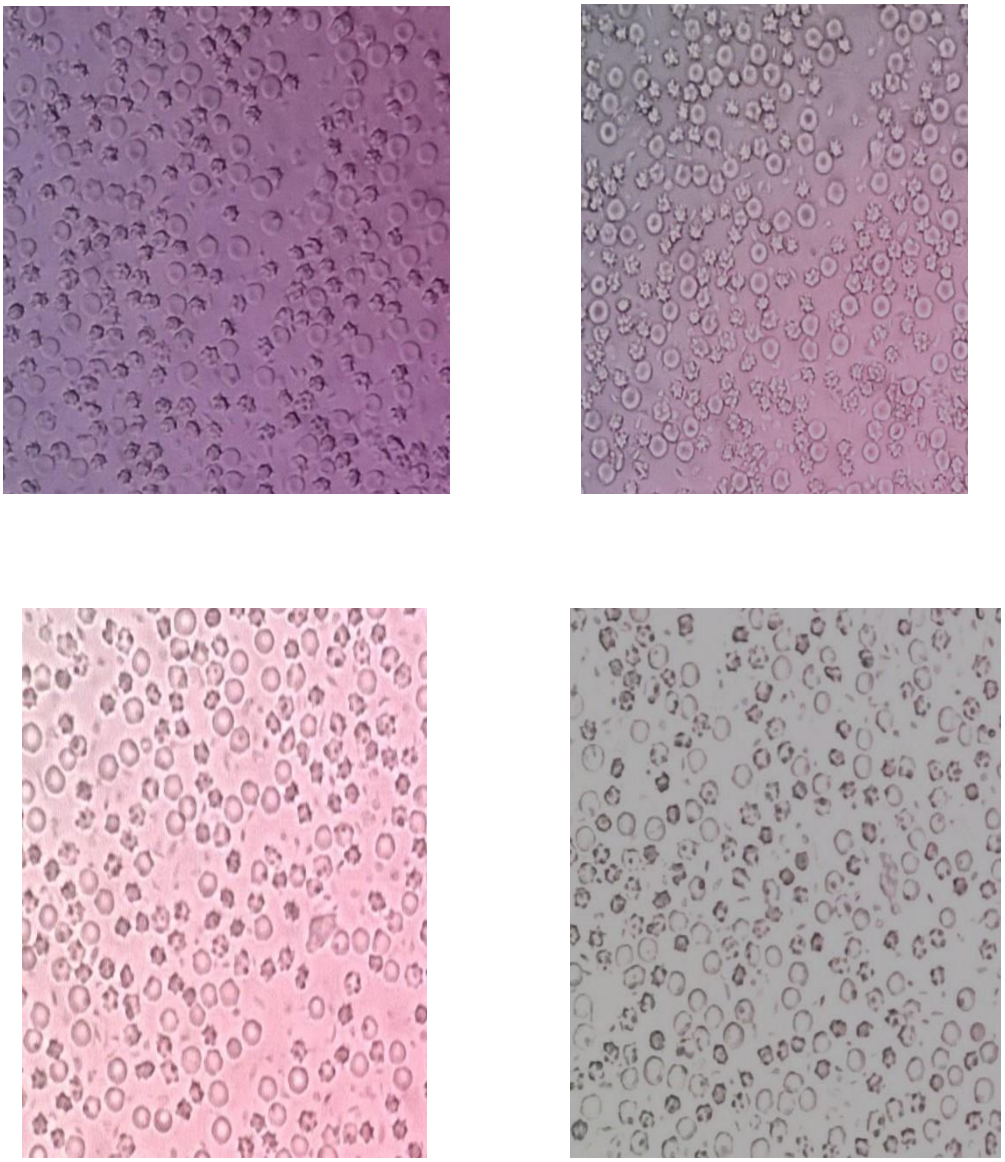
RBC MORPHOLOGY

Figure 11: Morphology of RBC at 0 day



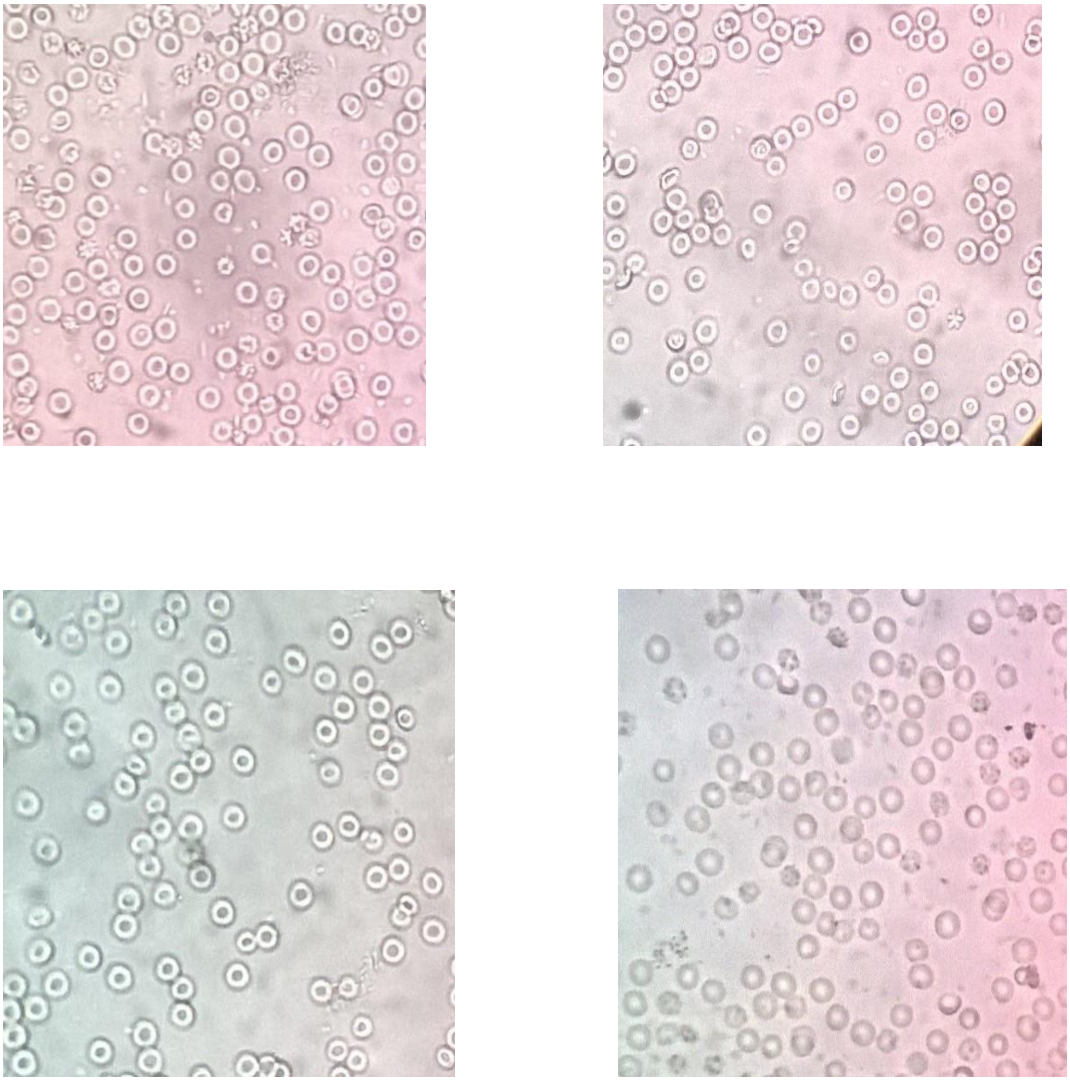
The morphology of RBC was observed on 0 day. All the cells are in same size and shape when focused under the microscope.

Figure 12: Morphology of RBC after the administration of Streptozotocin.



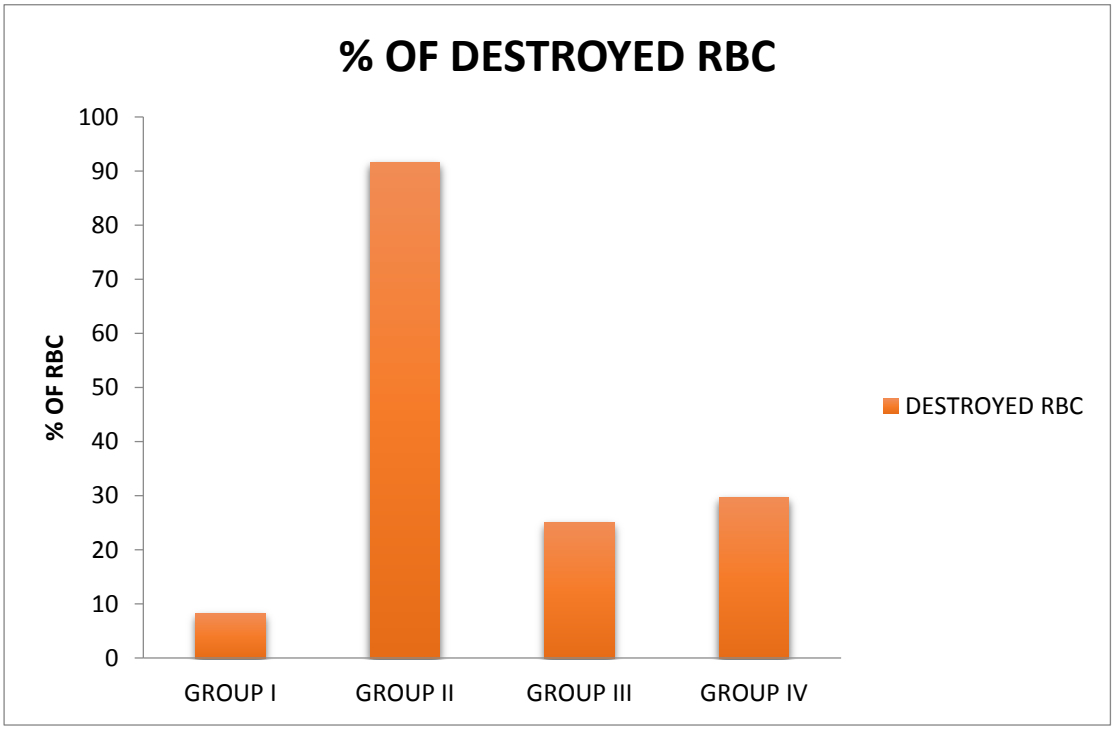
The morphology of RBC was observed on 7thday of Streptozotocin administration. Heinz bodies were observed with crenated edges and changes in the shape of red blood cells were observed.

Figure 13: Morphology of RBC after the administration of aqueous extract of *Phyllanthus emblica*L inn., for 42 days.



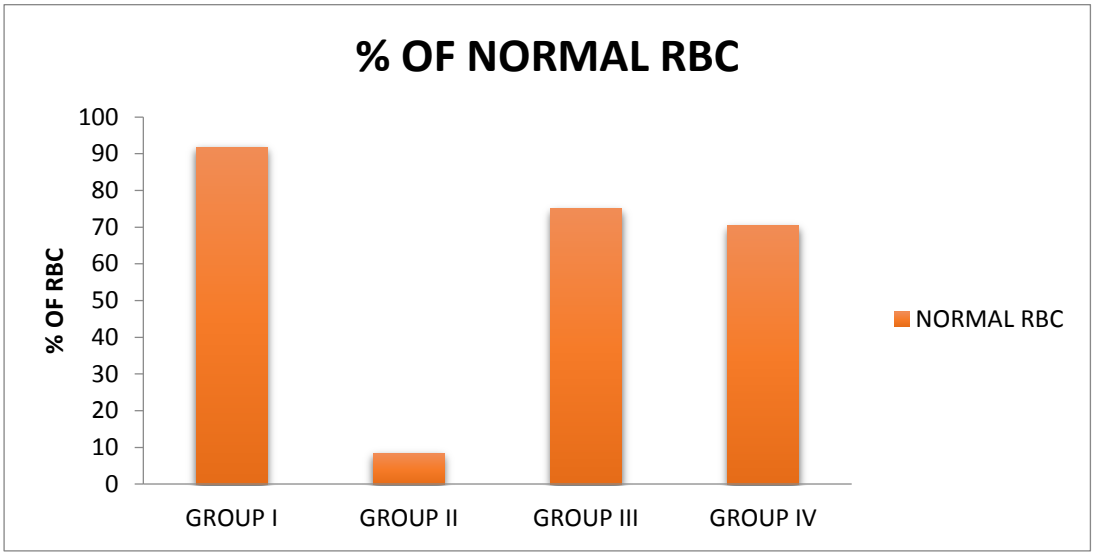
There is a reduction in the number of Heinz bodies after the administration of aqueous extract of *Phyllanthus emblica* Linn.for 42 days.

Figure 14: Percentage of destroyed RBC after the administration of Streptozotocin.



About 91% of red blood cells were affected with the administration of Streptozotocin and presence of Heinz bodies with crenated edges were observed.

Figure 15: Percentage of the normal RBC after the administration of aqueous extract of *Phyllanthus emblica* Linn. at the end of 42 days.



About 70% of Heinz bodies were treated with the administration of *Phyllanthus emblica* Linn., for 42 days and become to its normal shape and the results showed that *Phyllanthus emblica* prevents the destruction of RBC.

Discussion

7 DISCUSSION

Anaemia is considered as a major health problem throughout the world. Anaemia is a condition in which there is a deficiency of red blood cells or of haemoglobin in the blood, resulting in pallor and weariness. More than half of the world's population experience some forms of anaemia in their life time. The incidence of anaemia is higher in the third world than in developed countries[61].

Such prevalence has been attributed to various aggravating factors such as poor nutrition, pregnancy, high prevalence of blood parasites for example, plasmodium, trypanosome and helminthes infection. Prolonged use of non-steroidal anti-inflammatory drugs as well as exposure to toxic chemicals such as phenyl hydrazine have also been implicated to cause anaemia. Due to the high prevalence and possibility of even further increase, there is the need to prevent it or seek for more cost effective and better treatment strategies[61][65].

From ancient time, medicinal plants in Ayurveda are believed to be useful in strengthening the hematopoietic and immune system of an individual. Ayurvedic physicians suggested various herbs for the treatment of haematological disorders as a source of iron and other minerals[62][19]

Various researchers successfully evaluated the potential of several medicinal plants in the treatment of anaemia using various experimental animal models. *Ageratum conyzoides, Boerhavia diffusa, Centella asiatica, Hemide smusindicus, Ichnocarpus frutescens, Momordica charantia, Moringa oleifera, Phyllunthus amarus, Punica granatum, Ocimum tenuiflorum, Solanum americanum*are the some plants used in the treatment of anaemia[63][64][65][66][71].

*Phyllanthus emblica*is traditionally used in ayurveda to treat fever, inflammation peptic ulcer. Analgesic, digestive, hair tonic diuretic, anti tussive, cardio protective, chemo preventive, free radical scavenging activity, antimutagenic, cancer, antidiabetic[19][20].

The Phytochemical analysis of aqueous extract of fruits of *Phyllanthus emblica* revealed the presence of tannins, alkaloids, ascorbic acid, phyllembin, phyllemblic acid, gallicacid, ellagic acid and also minerals like iron and zinc [19].

To induce anaemia in the wistar rats are administered to Streptozotocin 45mg/kg by intraperitonially for seven days,[48]on the 8^{th} day the blood was collected and haematological studies are performed then the blood was centrifuged and the serum was separated and used for the RBC morphology studies.

HAEMATOLOGICAL STUDY

RBC count

The normal RBC count in wistar rat is $10.12\text{-}16.6X10^{12}$/L. After seven days of Streptozotocin administration the RBC count was reduced to $7.23X10^{12}$/L because of the destruction of RBC. The destruction of red blood cells is maybe due to the presence of oxidative stress[10]. This reduced RBC count was increased after the treatment of aqueous extract of *Phyllanthus emblica*.

Haemoglobin level (Hg)

There is a significant reduction in haemoglobin level of animals after the administration of Streptozotocin. The haemoglobin level is fall from 15.7 to 10.2g/DL. The reduced haemoglobin level shows the presence of anaemia in animals after the

administration of aqueous extract of *Phyllanthus emblica* Linn., the haemoglobin level is increased upto 13.6g/DL.

Hematocrit (HCT)

It is also called as packed cell volume. It is the volume of packed red cells obtained after centrifugation of sample of anti-coagulated venous or capillary blood. After the induction of anaemia the hematocrit was reduced to **38.13%** which is lower than the normal value and this reduction proved the presence of anaemia. There is a significant increase in PCV volume after treating the animals with aqueous extract of *Phyllanthus emblica* Linn., and the PCV was increased upto 41.26% .

Mean cell volume (MCV)

It is also called as mean corpuscular volume. It represents the average volume of a single red cell, and it expressed in femtolitres. There is a change in MCV volume in before and after the treatment with the *Phyllanthus emblica.* The reduced MCV volume by the administration of streptozotocin was improved by the *Phyllanthus emblica* Linn.,

Mean corpuscular haemoglobin (MCH)

It is the average amount of haemoglobin in each red cell and expressed in picograms. There is a low MCH volume is observed in negative control group by the administration of streptozotocin and results in microcytic hypochromic canaemia. This value is increased by the administration of aqueous extract of *Phyllanthus emblica* Linn.,

Mean corpuscular haemoglobin concentration (MCHC)

This represents the average concentration of haemoglobin in a given volume of packed red cells and it expressed in g/dL. Low MCHC values are observed in the negative control group and cause microcytic hypochromic anaemia. These low MCHC values are raised after giving the aqueous extract of *Phyllanthus emblica* Linn., to the animals.

Red cell distribution width (RDW)

It is the degree of variation of red cell size and can be determined on some blood cell analyzers. The comparision of negative and treatment group shows different values because the aqueous extract of *Phyllanthus emblica* Linn., increases the Red cell distribution width.

In this haematological study the reduced MCV, MCH, MCHC and RDW were observed, these reductions may be due to the oxidative stress caused by the administration of Streptozotocin and also there is a marked reduction in RBC count, Hg and HCT.This study also proves the presence of anaemia in Streptozotocin treated animal and this effect maybe due to oxidative stress. Administration of *Phyllanthus emblica* Linn., increases the RBC count, Haemoglobin level, Haematocrit, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration and Red Cell Distribution Width.

RBC MORPHOLOGY

The normal Red Blood Cells is in the shape of biconcave disk and the size of red blood cells is 6 to 9μm in diameter is 1.5 to 2.5μm thick. Normally red blood cells are relatively uniform in size and shape. Pathologic red cells may be larger or smaller than normal, may be abnormally shaped, or may contain inclusions.

Streptozotocin is a naturally occurring chemical that particularly toxic to the animals[48]. It induces changes in the red cell membrane, which result in oxidative denaturation of Hb. The effect of the denaturation is the formation of an altered Hb called "Heinz bodies" which reduces the life span of the erythrocytes[67][11]. Altered erythrocytes are removed by the spleen and liver of the reticulo endothelial system resulting in compensated haemolytic anemia[10][11].

Presences of Heinz bodies are observed in the red blood cells morphology after the administration of Streptozotocin in all group except the control group. The presence of Heinz bodies with crenated edges furthermore confirms the presence of hemolytic anaemia due to the oxidative stress caused by reactive oxygen species[68][69].

Heinz Bodies

Heinz Bodies is caused by destruction of haemoglobin and observed as inclusions. The Heinz Bodies has round, refractile inclusions found on the periphery of the cell[10]. It consists of denatured globin produced by the destruction of haemoglobin. They may occur due to thalassemia, drug induced anaemia, unstable haemoglobinopathies[12].

This study proves that Streptozotocin is able to cause anaemia in animals due to its oxidative stress. Appearance of Heinz bodies in red blood cell morphology proves the presence of anaemia and it maybe a type of hemolytic anaemia[70] [10].

It also contains phytochemical constituents such as flavonoids, tannins, and gallicacid. The value of zinc present in the fruits of *Phyllanthus emblica* Linn., is about 4ppm which has been reported to possess anti-anaemic potentials.[19].

There is a reduction in the percentage of Heinz bodies after the administration of aqueous extract of *Phyllanthus emblica* Linn., when compared with negative control group of animals . *Phyllanthus emblica* Linn., also increases the RBC count, Haemoglobin level, Haematocrit, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration and Red Cell Distribution Width. So this result was showed that fruits of aqueous extract of *Phyllanthus emblica*Linn., found to possessed anti-anaemic activity due to the presence of active constituents and also has been responsible for reducing oxidative stress.

Conclusion

8 CONCLUSION

Anaemia is considered to be one of the major health problems especially in chronic disorder like diabetes, rheumatoid arthritis, cancer, kidney disease, and HIV. According to the World Health Organization, about 30% of people throughout the world suffer from anemia. The most common cause of anaemia is iron deficiency.

In this present study it has been shown that the aqueous extract of *Phyllanthus emblica* Linn., increases the RBC count, Haemoglobin level, Haematocrit, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration and Red Cell Distribution Width and also improved the hemolytic anaemia induced by Streptozotocin in test animals, when compared with the untreated animals in the negative control group (Group 2).

On the basis of the above results the RBC morphological studies, reduction in the percentage of Heinz Bodies after the administration of aqueous extract of *Phyllanthus emblica* Linn.,when compared with negative control group (Group 2) of animals.

Hence it was suggested that the fruits of aqueous extract of *Phyllanthus emblica* Linn., possessed significant anti-anaemic effect. This may probably due to the anti-oxidant property of *Phyllanthus emblica* Linn.,

It was showed that *Phyllanthus emblica*Linn., contains natural antioxidant and possessed anti-anaemic activity which can be safely used in man. Further isolation and purification of bioactive compound from the fruits of *Phyllanthus emblica* may reveal the presence of potent novel anti-anaemic agent and also to unveil the molecular mechanism behind its therapeutic action.

Bibliography

BIBLIOGRAPHY

1. John P. Greer, Daniel A. Arber, BertilGlader, Alan F List, Robert T. Means. Jr, FrixosParaskeras, George M. Rodgers. Wintrobe's Clinical Hematology. 13th edition. Lippincott Williams & Wilkins; 2013. p. 587-589.
2. RamadasNayak, SharadaRai. Rapid Review on Haematology. Jaypee Brother Medical Publishers (P) Ltd.; 2014. p. 3-41.
3. John C. Aster, Olga Pozdynyakova, Jefferly L. Kutok. Haematopathology. Elsevier; 2012. p. 2-16.
4. Ashok Kumar CK, Revathi K, Mohanalakshmi S. A Review on Edible Herbs as Haematinics. International Journal of Pharmacy.2012;2(2): 44-53.
5. TanushreeSrivastava, HimanshuNegandhi, Sutapa B Neogi, Jyoti Sharmaand RenuSaxena. Methods for Haemoglobin Estimation. Journal of Hematology and Transfusion.2014; 3(4):213-218.
6. www.nobelprize.org/medicine/aqreats.
7. www.who.int/topics/Anaemia/in.
8. RenuSaxena, HpPati, M Mahapatra. Atlas of Haematology. 1st Edition. New Delhi:JaypeeBrother Medical Publishers (P) Ltd.; 2011. p.1-41.
9. KD Tripathi. Essentials of Medical Pharmacology. 5th edition. New Delhi: Jaypee Brother Medical Publishers (P) Ltd.; 2003. p.551-555.
10. Richard Harrey and Denise Jenifer. Biochemistry Lippincott's illustrated Reviews. 5th edition. New Delhi: McGraw Hill Publishers; 2011. p. 103, 153.
11. Irma Periara, Tracy L George, Daniel A Arber. Atlas of Peripheral Blood. New Delhi: McGraw Hill Publishers; 2012. p. 38-45.

12. Dacie Lewis. Blood Cell Morphology in Health and Disease. Practical Haematology. 9th edition. Kolkata: Hillton& Company;2011. p. 75.
13. B.G. Katzung, S.B. Masters, A.J. Trevor: Basic and clinical pharmacology. 11th edition. New Delhi: McGraw Hill Publishers; 2012. p.112-135.
14. www.rxlist.com
15. Eidensberg D M, Davis RB, Ettner SL, Appel S, wilkey S. Trends in alternative medicine used in united states. 1990-1997; Results of a follow up national survey. Journal of American Medical Association. 1998; 4(2): 1569-1575.
16. WHO, Regulatory situation of herbal medicine: A world wide review. Geneva: World Health Organisation. 2007.
17. WHO, General guidelines for methodologies on researchand evaluation traditional medicines. Geneva. World health organisation. 2009.
18. WHO Unicef, Anaemia Statement:Herbs as Haematinics. New York: World Health Organisation.2004.
19. MasumaZaki, Wajeeha Begum, Tabasum Ali Bhat, HeenaKausar, Amla (*EmblicaOfficinalis*). The Wonderful Unani Drug: A Review. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(9): 1560-1572.
20. VirendraYadav, BrijeshDuvey, Shobha Sharma and Babli Devi.Amla (*Emblicaofficinalis*) – Medicinal Food and Pharmacological Activity, International Journal of Pharmaceutical and Chemical Sciences. 2014;3(3): 601-619.
21. Malik Hassan Mehmood,Hasan SalmanSiddiqi,Anwarul Hassan Gilani. The antidiarrheal and spasmolytic activities of *Phyllanthusemblica* are mediated

through dual blockade of muscarinic receptors and Ca(2+) channels. Department of Biological & Biomedical Sciences.2011; 133(2):856-865.

22. Ragini Sharma, Neelam Jain, Deepika Rani, Amrita jaitawat, S. M. Kantwa, Role of *Emblicaofficinalis*and *Foeiniculumvulgare*during pregnancy and lactation. A Review International Journal of Advanced Multidisciplinary Research.(2015); 2(4): 103–113.
23. D. Madhavi, K. Rudrama Devi, K. Kesava Rao1 and P. Reddy. Modulating effect of Phyllanthus fruit extract against lead genotoxicity in germ cells of mice. Journal of Environmental Biology. 2007; 28(1): 115-117.
24. JeevangiSantoshkumar, Manjunath, SakharePranavkumar M. A Study of Anti-Hyperlipidemia, Hypolipedimic and Anti-Atherogenic Activity of Fruit of *EmblicaOfficinalis* (Amla) In High Fat Fed Albino rats. International Journal of Medical Research & Health Sciences.2013; 2(1):70-77.
25. Dang GK, Parekar RR, Kamat SK, Scindia AM, Rege NN.Anti-inflammatory activity of *Phyllanthusemblica, Plumbagozeylanica* and *Cyperusrotundus* in acute models of inflammation. Pubmed.2011;25(6):904-908.
26. Jose JK, Kuttan G, Kuttan R.Antitumour activity of *Emblicaofficinalis*. Pubmed. 2001; 75(2-3):65-69.
27. Vasudevan M, Parle M.Memory enhancing activity of Anwalachurna (*Emblicaofficinalis*Gaertn.): an Ayurvedic preparation.Pubmed.2007;91(1):46-54.
28. Biswas NR, Gupta SK, Das GK, Kumar N, Mongre PK, Haldar D, Beri S.Evaluation of Ophthalcare eye drops- a herbal formulation in the management of various ophthalmic disorders.Pubmed. 2001; 15(7):618-620.

29. Mai A Elobeid and Elham A Ahmed. Antidiabetic Efficacy of Aqueous Fruit Extract of Amla (*Emblicaofficinalis*, Gaertn) in Streptozotocin-Induced Diabetes Mellitus in Male Rats. Tropical Journal of Pharmaceutical Research 2015; 14(5): 801-806.
30. Pankaj R. Mali. Study of Antidiabatic Activity of *PhyllanthusEmblica*Linn. and*Curcuma Longa* Linn. on Alloxan induced mice. Science Journal. 2012; 1(2): 34-39.
31. Singh MK, Yadav SS, Gupta V and Kathtri S.Immunomodulatory role of *Emblicaofficinalis* in arsenic induced oxidative damage and apoptosis in thymocytes of mice.Pubmed. 2013; 27(13):191-193.
32. Colucci R, Dragoni F, Conti R, Pisaneschi L, Lazzeri L, Moretti S. Evaluation of an oral supplement containing *Phyllanthusemblica* fruit extracts, vitamin E and carotenoids in vitiligo treatment. Pubmed. 2015;28(1):17-21.
33. Costa A, Moisés TA, Cordero T, Alves CR, Marmirori J. Association of emblica, licorice and belides as an alternative to hydroquinone in the clinical treatment of melasma. Pubmed. 2010; 85(5):613-620.
34. Venil N. Sumantran, AsavariKulkarni, RuchaChandwaskar, AbhayHarsulkar.Chondroprotective Potential of Fruit Extracts of *Phyllanthusemblica* in Osteoarthritis. Journal of Evidence Based Complementary and Alternative Medicine.2008; 5(3): 329-335.
35. Rao TP, Okamoto T, Akita N, Hayashi T*et al.*Amla (*Emblicaofficinalis*Gaertn.) extract inhibits lipopolysaccharide-induced procoagulant and pro-inflammatory factors in cultured vascular endothelial cells. Pubmed. 2013;110(12):2201-2206.

36. Mehmood MH, Rehman A, Rehman NU, Gilani AH.Studies on prokinetic, laxative and spasmodic activities of *Phyllanthusemblica* in experimental animals. Pubmed. 2013; 27(7): 1054-60.

37. Jindal A, Soyal D, Sharma A, Goyal PK. Protective effect of an extract of *Emblicaofficinalis* against radiation induced damage in mice. Pubmed.2008; 3(7): 98-105.

38. Hema Sharma Datta, Shankar Kumar Mitra and BhushanPatwardhan. Wound Healing Activity of Topical Application Forms Based on Ayurveda. Journal of Evidence Based Complementary and Alternative Medicine. 2011; 20(11): 90-95.

39. Alam MI, Gomes A. Snake venom neutralization by Indian medicinal plants (Vitexnegundoand *Emblica officinalis*) root extracts. Journal of Ethanopharmacology.2003; 86(1): 75-80.

40. Golechha M, Bhatia J, Ojha S, Arya DS. Hydroalcoholic extract of *Emblica officinalis* protects against kainic acid-induced status epilepticus in rats: evidence for an antioxidant, anti-inflammatory, and neuroprotective intervention. Pubmed.2011; 49(11): 1128-1136.

41. Sayed S, Ahsan N, Kato M, Ohgami N, Rashid A, Akhand AA. Protective effects of *phyllanthusemblica* leaf extract on sodium arsenite-mediated adverse effects in mice.Pubmed. 2015;77(1): 145-153.

42. SwethaDasaroju, Krishna Mohan Gottumukkala. Current Trends in the Research of *Emblicaofficinalis*(Amla): A Pharmacological Perspective. International Journal of Pharmaceutical science. 2014; 24(2): 150-159.

43. SankaranMirunalini, VelusamyVaithiyanathan and Mani Krishnaveni., Amla: A Novel Ayurvedic Herb as A Functional Food for Health Benefits"- A Mini

Review. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(1): 45-52.

44. Mrs.Manali. M. Bhid, Mr. Sachin. A. NativeRoles of*EmblicaOfficinalis* (Amla) In Medicine World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(6): 604-615.
45. Tripathi Rakesh Kumar and Dwivedi S.N.Phenological and ethnobotanical studies of *Emblicaofficinalis*Gaertn. International Journal of Pharmacy & Life Sciences. 2010; 1(6):340-342.
46. Jitin Rahul.An ethnobotanical study of Medicinal Plants in Taindol Village, District Jhansi, Region of Bundelkhand, Uttar Pradesh, India. Journal ofMedicinal Plants Studies. 2013; 1(5):59-71.
47. Ghosh MN. Fundamentals of Experimental Pharmacology.5th edition. Kolkata: Hilton &company; 2011. p. 4-22.
48. http://www.drugs.com.
49. Abeer A. Abd El-Baky.Clinicopathological Effect of Extract *Camellia sinensis*on Streptozotocin-induced diabetes in rats. World Journal of Medical Science. 2013; 8(3): 205-213.
50. IAUCU, Guidelines for blood collection. AUCU Review. 2015.
51. S Parasuraman, R Raveendran and R Kesavan. Blood sample collection in small laboratory animals. Journal of Pharmacology and Pharmacotherapeutics.2010; 1(2): 87-93.
52. http//www.sysmex.co.in.
53. www.rn.org.

54. Mauro Buttarello, MD, and Mario Plebani. Automated Blood Cell Counts. Asian Journal of Chemistry and Pharmaceutical Science.2016; 2(3): 214-219.
55. AyalewTefferi, Curtis A. Hanson and David J. Inwards. How to interpret and pursue an abnormal Complete Blood Cell Count in adults. 2005;80(7):923-936.
56. Anne MV, Debra JP, Mickkey LB. Laboratory Diagnostic Tests. 5th edition. 2013; 190-200.
57. Shrish M. Kawthalkar. Essentials of Haematology. 2nd edition. New Delhi:Jaypee Brothers Medical Publishers (P) Ltd.; 2013. p. 52-219.
58. Gillian Rozenberg. Cases in Microscopic Haematology. 1stEdition. Elsevier; 2011; p. 1-19.
59. Shauna C. Anderson Young, Keila B. Poulson. Anderson's Atlas of Haematology. 2nd Edition. New Delhi:Lippincott Williams & Wilkins. 2013; p. 18-51.
60. https://theartosmed.wordpress.com.
61. AshishTuraskar, Sachin More, Rizwan Sheikh, J. Gadhpayle*et al.*Antianaemic Potential of *Swertiachirata*on Phenylhydrazine induced reticulocytosis in rats. Asian Journal of Pharmaceutical Science 2013; 2(1):37-41.
62. PrasunaSundariPingali, PrathimaSrinivas, Madhava Reddy B.Study of Anti-Anaemic Effect of*SchreberaSwietenioides*Roxb. in rat models. Asian Journal of Pharmaceutical and Clinical Research.2015; 8(5): 36-39.
63. Purnima A, VamseeVeena A, Renuka Sand Rajendra R. Comparative anti anaemic activity of *Murrayakoenigii*(Linn.) Spreng leaves and itscombination with *Emblicaofficinalis*. 2011; 3(3): 216-219.
64. Aluminium chloride induced anaemia using rodents. International Journal of Advanced Research in medical science.2014; 1(1): 45-49.

65. MacDonald Idu ,GoddiditIgbafe , Joseph Erhabor. Anti-anaemic activity of *Jatrophatanjorensis*Ellis &Saroja in Rabbits. Journal of Medicinal Plants Studies. 2014; 2(1): 64-72.
66. VamseeVeena A, Jyothi Y, RinaPokhrel, RajdwipGhosh. Comparative anti anemic activity of *Azadirachtaindica*leaves and its combination with *Emblicaofficinalis*in phenyl hydrazine induced anemia using rats. Journal of chemical and Pharmaceutical research.2015; 7(8):1019-1022.
67. R. Guitart, C. Mateu, A Lopez i Agullo, J. AlberolaHeinz body anaemia in two dogs after Catalan spring onion ("calcot") ingestion: a case reports. VeterinarniMedicina. 2008; 53(7): 392–395.
68. Fred L. Metzger Jr, Alan Rebar DVM. Evaluating Blood Flims.VeterinarniMedicina. 2004; 53(7): 192–197
69. M. M. Christophejr,G. White,A Nd J. W. EatoErythrocyte Pathology And Mechanisms Of Heinz Body-Mediated Hemolysis In Cats. Veterinary pathobiology. 1990; 27:299-310.
70. GurpreetDhaliwal, Patricia A, Cornett Mand Lawrence M. Hemolytic Anaemia Concise review of Hemolytic Anaemia. New Delhi: McGraw Hill Publishers;2004.
71. Ashok Kumar CK, Revathi K, Mohanalakshmi S,A Review on Edible Herbs as Haematinics. International Journal of Pharmacy, 2012;2(2): 44-53.